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Single-Laboratory Evaluation of Method 8080 -Organochlorine Pesticides and PCBs (Polychlorinated Biphenyls)

Acurex Corp., Mountain View, CA

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SINGLE-LABORATORY EVALUATION OF METHOD 8080 -- ORGANOCHLORINE PESTICIDES AND PCBs

by

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16. ABSTRACT

The design, execution and results of the improvement and single-laboratory evaluation of SW-846 Method 8080, "Organochlorine Pesticides and PCBs", are described. In this study, the application of this method to the analysis of aqueous and solid samples for organochlorine pesticides and PCBs was examined. Deficiencies of the present method were corrected by substituting a silica gel extract cleanup procedure for the original Florisil procedure (which results in a better separation of the pesticides from the PCBs) and by using capillary gas chromatographic columns in place of the packed columns (which provides a better gas chromatographic separation). A limited evaluation of the sample extraction methods 3510, 3520, 3540 and 3550 preceded this study. Ruggedness testing of some of the more important parameters of the modified method was conducted to determine the effect of critical parameters on method performance. Method performance parameters, including precision, accuracy and detection limit data, are presented and discussed. The limitations of the method are described and suggestions are included on how to put this method to best use. In addition to the revised protocol, the report includes an extensive literature review covering analytical methods for the determination of organochlorine pesticides and polychlorinated biphenyls in water, soil, sediment and sludge samples. The revised method is recommended for use as part of the Agency's monitoring program.

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PREFACE

This is the final report for Work Assignments 5 and 8, EPA Contract 68-03-3226, "Evaluation of GC Method for Organochlorine Pesticides and PCBs," conducted at Acurex Corporation, Project Nos. 7902 and 8001. The project was directed by Dr. Viorica Lopez-Avila. The work was performed in the Environmental Systems Division.

This report was written by Dr. Viorica Lopez-Avila. Technical support for the project was provided by Dr. Sarah Schoen and Mr. June Milanes.

ABSTRACT

Method 8080 was developed for the determination of certain organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in liquids and solids. Liquid samples are extracted according to Method 3510 (separatory funnel) or Method 3520 (continuous liquid-liquid extractor) and solid samples according to Method 3540 (Soxhlet extraction) or Method 3550 (sonication), the extracts are concentrated, fractionated on Florisil and the fractions analyzed by gas chromatography (GC) on packed columns.

The Method 8080 protocol has been evaluated in a single laboratory on actual and simulated wastes. It was found that the Florisil cleanup method is problematic when both OCPs and PCBs are present; a cleanup and fractionation on deactivated silica gel is more advantageous. Sulfur in extracts can be removed with tetrabutylammonium sulfite. Toxaphene and chlordane pose special problems because of their multiple-peak responses. The use of capillary columns instead of packed columns in the GC analysis is advantageous because better separations are obtained for complex samples containing combinations of OCPs, PCBs, and other organics.

The Method 8080 protocol was revised, and several features were added or changed, including the cleanup and fractionation procedures which now specify deactivated silica gel, and the use of capillary columns instead of packed columns. The revised protocol was evaluated by using extracts of environmental samples spiked with the substances of interest of known concentrations. The precision and accuracy results indicate that the revised Method 8080 can be reliably applied to the determination of organochlorine pesticides and PCBs in liquid and solid matrices.

Included in this report as an appendix is an extensive literature review covering analytical methods for the determination of organochlorine pesticides and PCBs in water, soil, sediment and sludge samples.

TABLE OF CONTENTS

Section	Page
Preface	iii
Abstract	iv
Figures	vii
Tables	x
1. Introduction	1
2. Conclusions	4
3. Recommendations	6
4. Literature Review	7
5. Method 8080 Evaluation	10
5.1 Experimental	11
5.2 Results	11
6. Method Improvement	18
6.1 Extraction	18
6.1.1 Extraction of Aqueous Samples	18
6.1.2 Extraction of Soil Samples	22
6.1.3 Ruggedness Test for Method 3550	26
6.2 Extract Cleanup	29
6.2.1 Silica Gel Cleanup	29
6.2.2 Alumina Cleanup	36
6.2.3 Silica Gel/Celite	48
6.2.4 Florisil/Charcoal	49
6.2.5 Sulfur Cleanup	54
6.3 Gas Chromatography	55
6.4 Recommendations for Method Revision	73
7. Revised Method 8080 -- Evaluation Studies	74
7.1 Reproducibility Study for Gas Chromatographic Analysis	74
7.2 Method Precision and Accuracy	80
7.3 Method Detection Limit	97

CONTENTS (Continued)

Section Page

References. 100

Appendices

A. Literature Review. 102
B. Method 8080 - Organochlorine Pesticides and PCBs 191
C. Method 3510, 3520, 3540, 3550 Protocols. 221

FIGURES

Number Page

1 GC/EC chromatograms of the Florisil Fractions I, II, and III of liquid waste No. 1 extract, analyzed on the 1.8 m x 2 mm ID 1.5-percent OV-17/1.95-percent OV-210 column; isothermal at 190°C 14

2 GC/EC chromatograms of the Florisil Fractions I, II, and III of liquid waste No. 1 extract, spiked with the organochlorine pesticides and PCBs, and analyzed on the 1.8 m x 2 mm ID 1.5-percent OV-17/1.95-percent OV-210 column; isothermal at 190°C 15

3 GC/EC chromatograms of the Florisil Fractions I, II, and III of liquid waste No. 1 extract, analyzed on the 30 m x 0.25 mm ID DB-5 fused-silica capillary column. Temperature program: 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; helium at 16 psi. 16

4 GC/EC chromatograms of the Florisil Fractions I, II, and III of liquid waste No. 1 extract, spiked with the organochlorine pesticides and PCBs, and analyzed on the 30 m x 0.25 mm ID DB-5 fused-silica capillary column. Temperature program: 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; helium at 16 psi. 17

5 GC/EC chromatograms of the silica gel Fractions I, II, and III of liquid waste No. 1 extract, analyzed on the 30 m x 0.25 mm ID DB-5 fused-silica capillary column. Temperature program: 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; helium at 16 psi. 41

6 GC/EC chromatograms of the silica gel Fractions I, II, and III of liquid waste No. 1 extract, spiked with the organochlorine pesticides and PCBs, and analyzed on the 30 m x 0.25 mm ID DB-5 fused-silica capillary column. Temperature program: 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; helium at 16 psi. 42

FIGURES (Continued)

Number Page

7 GC/EC chromatogram of the liquid waste No. 1 extract after alumina cleanup (10 mL hexane). 45

8 GC/EC chromatogram of the liquid waste No. 1 extract after alumina cleanup (20 mL hexane). 46

9 GC/EC chromatogram of the liquid waste No. 1 extract after alumina cleanup (50 mL hexane). 47

10 GC/EC chromatogram of the liquid waste No. 1 extract after cleanup on alumina deactivated with 15 percent water. 50

11 GC/EC chromatogram of the liquid waste No. 1 extract after cleanup on alumina deactivated with 19 percent water. 51

12 GC/EC chromatogram of the sulfur standard before reaction with the TBA-sulfite reagent. 58

13 GC/EC chromatogram of the sulfur standard after reaction with the TBA-sulfite reagent 59

14 GC/EC chromatograms of the organochlorine pesticide standards A and B and of a composite of A and B, obtained on the 1.5-percent OV-17/1.95-percent OV-210 column. Concentrations: standard A, 0.05 to 0.10 ng/μ; standard B, 0.05 to 0.50 ng/μL; standard A and B composite, 0.05 to 0.10 ng/μL; isothermal at 190°C. 64

15 GC/EC chromatograms of a mixture of PCB 1016/1260 at 0.05 ng/μL and of a composite of organochlorine pesticide standards A and B and PCB-1016/1260 at 0.05 ng/μL, obtained on the 1.5-percent OV-17/1.95-percent OV-210 column; isothermal at 190°C 65

16 GC/EC chromatograms of a PCB-1016/1260 mixture at 0.5 ng/μL and of a composite of organochlorine pesticide standards A and B and PCB-1016/1260 at concentrations of 0.05 to 0.50 ng/μL and 0.50 ng/μL, respectively. The analyses were performed on the 1.5-percent OV-17/1.95-percent OV-210 column; isothermal at 190°C. 66

FIGURES (Continued)

Number Page

17 GC/EC chromatograms of the toxaphene standard at 0.10 ng/μL and of a composite of organochlorine pesticide standards A and B at 0.05 to 0.50 ng/μL and toxaphene at 0.10 ng/μL, obtained on the 1.5-percent OV-17/1.95-percent OV-210 column; isothermal at 190°C 67

18 GC/EC chromatograms of the toxaphene standard at 1.0 ng/μL and of a composite of organochlorine pesticide standards A and B at 0.05 to 0.50 ng/μL and toxaphene at 1.0 ng/μL, obtained on the 1.5-percent OV-17/1.95-percent OV-210 column; isothermal at 190°C 68

TABLES

Number		Page
1	Compounds Listed in Method 8080	2
2	Results of the Florisil Evaluation Study	12
3	Evaluation of Method 3510 Using Distilled Water Samples	19
4	Evaluation of Method 3520 Using Distilled Water Samples	20
5	Evaluation of Method 3540 Using Sandy Loam Samples	23
6	Evaluation of Method 3550 Using Sandy Loam Samples	24
7	Combinations of 7 Parameters Recommended for Testing the Ruggedness of Method 3550	26
8	Ruggedness Test for Method 3550 -- Recovery Data for the Organochlorine Pesticides	28
9	Ruggedness Test -- Group Differences for the Organochlorine Pesticides	29
10	Ruggedness Test -- Percent Recoveries of the Organochlorine Pesticides as a Function of Solvent Composition	30
11	Distribution and Percent Recoveries of the Organochlorine Pesticides and PCBs in the Silica Gel Column Fractions	32
12	Distribution and Percent Recoveries of the Organochlorine Pesticides and PCBs Spiked into the Distilled Water Extracts (Spike Level is 500 ng for Pesticides and 5,000 ng for PCBs)	33
13	Distribution and Percent Recoveries of the Organochlorine Pesticides and PCBs Spiked into the Liquid Waste No. 1 Extract (Spike Level is 500 ng for Pesticides and 5,000 ng for PCBs)	34

TABLES (Continued)

Number Page

14 Distribution and Percent Recoveries of the Organochlorine Pesticides and PCBs Spiked into the Liquid Waste No. 1 Extract (Spike Level is 5,000 ng for Pesticides and 50,000 ng for PCBs) 35

15 Distribution and Percent Recoveries of the Organochlorine Pesticides and PCBs Spiked into the Liquid Waste No. 2 Extract (Spike Level is 500 ng for Pesticides and 5,000 ng for PCBs) 36

16 Distribution and Percent Recoveries of the Organochlorine Pesticides and PCBs Spiked into the Liquid Waste No. 2 Extract (Spike Level is 500 ng for Pesticides and 50,000 ng for PCBs) 37

17 Distribution and Percent Recoveries of the Organochlorine Pesticides and PCBs Spiked into the Sandy Loam Extract (Spike Level is 500 ng for Pesticides and 5,000 ng for PCBs) 38

18 Distribution and Percent Recoveries of the Organochlorine Pesticides and PCBs Spiked into the NBS Sediment Extract (Spike Level is 500 ng for Pesticides and 5,000 ng for PCBs) 39

19 Distribution and Percent Recoveries of the Organochlorine Pesticides and PCBs Spiked into the Solid Waste Extract (Spike Level is 500 ng for Pesticides and 5,000 ng for PCBs) 40

20 Recovery of Selected Organochlorine Pesticides After Chromatography on Neutral Super I Woelm Alumina 43

21 Recovery of Organochlorine Pesticides Spiked into Liquid Waste No. 1 Extract and Subjected to Alumina Cleanup. 43

22 Recovery of the Organochlorine Pesticides Spiked into Liquid Waste No. 2 and Subjected to Alumina Cleanup 48

23 Alumina Cleanup Recoveries. 49

TABLES (Continued)

Number Page

24 Elution Patterns and Percent Recoveries from the Silica Gel/Celite Column Cleanup 52

25 Elution Patterns and Percent Recoveries from the Florisil/Charcoal Cleanup. 53

26 Recoveries of the Organochlorine Pesticides and PCBs Subjected to the TBA-Sulfite Procedure. 56

27 Recoveries of the Organochlorine Pesticides Spiked into Various Matrices and Subjected to the TBA-Sulfite Procedure 57

28 Summary of Organochlorine Pesticide Retention Times 60

29 Gas Chromatographic Conditions for the Determination of Pesticides and PCBs by GC/EC Using a 1.5-Percent OV-17/1.95-Percent OV-210 Column. 61

30 Gas Chromatographic Conditions for the Determination of Pesticides and PCBs by GC/EC Using a 3-Percent OV-1 Column. 61

31 Gas Chromatographic Conditions for the Determination of Pesticides and PCBs by GC/EC Using a DB-5 Fused-Silica Capillary Column. 62

32 Gas Chromatographic Conditions for the Determination of Pesticides and PCBs by GC/EC Using an SPB-608 Fused-Silica Capillary Column 63

33 Organochlorine Pesticides and PCBs Average Recoveries from Silica Gel Chromatography, Determined on an OV-17/OV-210 Column. 69

34 Organochlorine Pesticides and PCBs Average Recoveries from Silica Gel Chromatography, Determined on a 3-percent OV-1 Column. 70

TABLES (Continued)

Number Page

35 Average Recoveries from Silica Gel Chromatography, Determined on a 30 m x 0.25 mm ID DB-5 Fused-Silica Capillary Column . . . 71

36 Average Recoveries from Silica Gel Chromatography, Determined on an SPB-608 Fused-Silica Capillary Column 72

37 Reproducibility of the Response Factors of the Organochlorine Pesticides for 10 Consecutive Replicate Injections. 75

38 Reproducibility of the Retention Times of the Organochlorine Pesticides for 10 Consecutive Replicate Injections. 76

39 Reproducibility of the Response Factors for 10 Consecutive Injections of PCB-1016/1260 Standards 77

40 Reproducibility of the Area Response for 10 Consecutive Injections of PCB-1016/1260 Standards (Six Major Components). . 78

41 Reproducibility of the Retention Times of Six Major Components in a PCB-1016/1260 Mixture for 10 Consecutive Injections. . . . 79

42 Elution Patterns and Average Recoveries of the Organochlorine Pesticides and PCBs after Silica Gel Chromatography (Standards Only) 82

43 Elution Patterns and Average Recoveries of the Organochlorine Pesticides and PCBs after Silica Gel Chromatography (Liquid Waste No. 1 Extract) 85

44 Elution Patterns and Average Recoveries of the Organochlorine Pesticides and PCBs after Silica Gel Chromatography (NBS Sediment Extract) 88

45 Elution Patterns and Average Recoveries of the Organochlorine Pesticides and PCBs after Silica Gel Chromatography (Sandy Loam Extract) 91

TABLES (continued)

Number		Page
46	Elution Patterns and Average Recoveries of the Organochlorine Pesticides and PCBs after Silica Gel Chromatography (Soil Extract Contamination with Toxaphene)	94
47	Results of the Method Detection Limit Determination for Organochlorine Pesticides and PCBs in Distilled Water	98
48	Results of the Method Detection Limit Determination for Organochlorine Pesticides and PCBs in Sandy Loam Soil	99

SECTION 1

INTRODUCTION

The Environmental Monitoring Systems Laboratory in Las Vegas, Nevada, Environmental Protection Agency (EPA), has been responsible for evaluating some of the methods published in "Test Methods for Evaluating Solid Waste -- Physical Chemical Methods" (SW-846) for use with materials characteristic of the wastes regulated under the Resource Conservation and Recovery Act (RCRA). One of these methods is Method 8080, entitled "Organochlorine Pesticides and PCBs," which is used in the determination of chlorinated pesticides and polychlorinated biphenyls (PCBs) (Table 1) in ground water and in both liquid and solid matrices.

Acurex, under contract to the EMSL-LV, has been involved in the evaluation of Method 8080. The first phase of the study addressed the fractionation of the organochlorine pesticides and PCBs by Florisil chromatography and the feasibility of substituting the Florisil fractionation procedure with a more efficient method because the organochlorine pesticides are poorly separated from the PCBs and toxaphene. Also, the gas chromatographic determination of the organochlorine pesticides and PCBs using packed and capillary columns was evaluated, and a literature review of the analytical methodologies for the determination of the compounds listed in Method 8080 was conducted. Subsequent experiments, performed in the second phase, addressed the sample extraction procedures that are recommended in Method 8080: separatory-funnel extraction and continuous liquid-liquid extraction for aqueous samples, and Soxhlet extraction and sonication for solids (Methods 3510, 3520, 3540, and 3550, respectively). In parallel, experiments were conducted to develop a fractionation scheme using either silica gel or alumina chromatography or a combination of either silica gel/Celite or Florisil/charcoal chromatography. Upon completion of this phase, a revised protocol was prepared which was then evaluated in the third phase.

The development of the revised method focused on improving both the separation of the organochlorine pesticides from the PCBs prior to the gas chromatographic analysis and their identification by using the resolving power of a capillary column instead of that of a packed column. The analytical scheme given in the revised Method 8080 protocol employs silica gel fractionation (silica gel deactivated with 3.3 percent water). Four fractions are collected: Fraction I eluted with 80 mL hexane, Fraction II eluted with 50 mL hexane, Fraction III eluted with 15 mL methylene chloride, and Fraction IV eluted with 50 mL ethyl acetate. The determination of the organochlorine pesticides and PCBs which are recovered in these four fractions is performed by gas chromatography on a fused-silica capillary column and with electron capture detection.

TABLE 1. COMPOUNDS LISTED IN METHOD 8080

Parameter	Storet No.	CAS No.
alpha-BHC	39337	319-84-6
beta-BHC	39338	319-85-7
gamma-BHC (Lindane)	39340	58-89-9
delta-BHC	34259	319-86-8
Heptachlor	39410	76-44-8
Aldrin	39330	309-00-2
Heptachlor epoxide	39420	1024-57-3
gamma-Chlordane	39350	57-74-9
Endosulfan I	34361	959-98-8
4,4'-DDE	39320	72-55-9
Dieldrin	39380	60-57-1
Endrin	39390	72-20-8
Endosulfan II	34356	33212-65-9
4,4'-DDD	39310	72-54-8
Endrin aldehyde	34366	7421-93-4
Kepone	39018	143-50-0
Endosulfan sulfate	34351	1031-07-8
4,4'-DDT	39300	50-29-3
4,4'-Methoxychlor	NA	72-43-5
Toxaphene	39400	8001-35-2
PCB-1016	34671	12674-11-2
PCB-1221	39488	1104-28-2
PCB-1232	39492	11141-16-5
PCB-1242	39496	53469-21-9
PCB-1248	39500	12672-29-6
PCB-1254	39504	11097-69-1
PCB-1260	39508	11096-82-5

NA -- Storet number not available at the time of report preparation.

The revised protocol was evaluated in Phase III by using extracts of environmental samples spiked with the substances of interest at known concentrations. The evaluation studies were conducted at three concentrations, each in triplicate. The precision and accuracy results indicated that the revised Method 8080 could be reliably applied to the determination of the organochlorine pesticides and PCBs in liquid and solid matrices. A method detection limit determination was performed for both the aqueous and the solid matrices.

SECTION 2

CONCLUSIONS

Based on the results presented in this report, the following conclusions can be drawn concerning the determination of the organochlorine pesticides and PCBs:

- A revision of Method 8080 for the determination of the organochlorine pesticides and PCBs was necessary because the Florisil procedure does not allow separation of the organochlorine pesticides from the multi-component PCB mixture (except for endosulfans and endrin aldehyde) and because the packed column does not have enough resolving power to handle complex environmental samples.

- The revised Method 8080 presented in Appendix B has been evaluated in a single laboratory with a few relevant liquid and solid wastes. The utilization of silica gel fractionation and capillary column analysis was found to be appropriate. When silica gel fractionation was used, four fractions were collected. The silica gel procedure is tedious and does account for a major part of the analysis time. However, we have demonstrated that the method precision is better than ±20 percent for all compounds, and the accuracy is greater than 60 percent when standards are processed through the silica gel procedure. Fraction IV may be combined with Fraction III in those cases when Kepone is not expected to be present and when the matrix is not very complex, and thus may reduce the number of analyses per sample. Furthermore, the capillary column gas chromatographic analysis allows complete separation of the five organochlorine pesticides that elute with the PCBs in Fraction I. The use of a second capillary column as a confirmatory column is recommended although the presence of Kepone cannot be confirmed with this column.

- The detection limits of the revised method for water samples range from 0.023 to 0.086 pg/L for the organochlorine pesticides and from 0.54 to 0.90 pg/L for the PCBs. The method detection limits for solid samples is higher, ranging from 1.1 to 5.7 ug/Kg for the organochlorine pesticides and from 57 to 70 pg/Kg for the PBCs. These values are representative of clean samples. The actual method detection limits should be determined for each matrix.

- Toxaphene, if present in the sample at concentrations 10 times as high as the organochlorine pesticides, is likely to cause problems in the determination of the organochlorine pesticides and PCBs since it does not elute from the silica gel column in a narrow band. Other analytical techniques (e.g., chemical ionization mass spectrometry) should be investigated for the determination of toxaphene.

SECTION 3

RECOMMENDATIONS

The revised Method 8080 presented in this report has been evaluated in a single laboratory by using a few relevant liquid and solid wastes. However, the revised method should be evaluated in a number of different laboratories on additional sample types. This would help identify the applicability range of the method and define its interlaboratory method performance.

When the revised Method 8080 is applied to samples which have not been previously characterized, confirmation of compounds identified in the sample may be required. Although the revised Method 8080 specifies a second capillary column for confirmation, additional work should be performed to determine how reliable the identification obtained by the multi-column technique really is. Chemical derivatization techniques may prove helpful in those cases where fewer compounds need to be confirmed. Furthermore, determination of the organochlorine pesticides and PCBs by gas chromatography/mass spectrometry should be considered for future investigation since it would certainly reduce the amount of work required to fractionate a sample extract, to analyze fractions, and to confirm compound identifications.

The use of internal standards and surrogate spiking compounds needs to be investigated. Much of the variability inherent to trace analysis in difficult samples can be reduced by the use of internal standards. Likewise, surrogate spiking compounds can provide recovery information for each sample. These surrogate spiking compounds should be selected such that they will elute into each of the four fractions collected from the silica gel column.

SECTION 4

LITERATURE REVIEW

In the initial phase of this study, a literature review covering analytical methods for the determination of the organochlorine pesticides and polychlorinated biphenyls (PCBs) in water, soil, sediment, and sludge samples was conducted. This literature review was performed by using the Computerized Chemical Abstracts search as well as several review books dealing specifically with the analysis of organochlorine pesticides and PCBs. Furthermore, recent issues of Analytical Chemistry, the Journal of Chromatography, the Journal of Agricultural and Food Chemistry, the Journal of the Association of Official Analytical Chemists, and the Environmental Science and Technology were searched to gather recent references that were not yet in the computer data bases.

The computer searches were done by using DIALOG. Chemical Abstracts files were searched back to 1972 for all references containing "pesticides," "PCBs," "gas chromatography," "extraction," and "cleanup." These searches printed out approximately 500 citations of which approximately 70 were judged relevant to the objectives of this study.

Two books, "Analysis of Pesticides in Water," Volumes I and II, by Alfred S. Y. Chau and B. K. Afghan, CRC Press, 1982, and "Analysis of Pesticide Residues," edited by H. Anson Moye, John Wiley & Sons, 1981, have been thoroughly reviewed for additional information and citations.

The literature review summary is included in Appendix A in the following order:

- Sample preservation techniques
- Extraction techniques for organochlorine pesticides and PCBs from water and soil (type of extraction technique, solvent, factors that affect recovery)
- Cleanup techniques
- Gas chromatographic analysis (column, retention time information, chromatography problems, etc.)
- Compound confirmation (chemical derivatization techniques)
- Stability of organochlorine pesticide solutions.

From the literature review presented in this report, it is evident that there is sufficient information in the literature on sample extraction, cleanup, and analysis for organochlorine pesticides and PCBs to form a data base upon which to design future experiments. However, none of the information retrieved discusses specifically how to determine all of organochlorine pesticides and PCBs listed in Method 8080 in hazardous liquid and solid samples.

Appendix A was prepared in the summer of 1985; since then a few additional relevant studies on this subject have been published and are worth mentioning (1-4). One such study deals with the determination of PCBs in environmentally contaminated sediments (1). Three sediment samples contaminated with PCBs were analyzed by ten laboratories using standardized procedures for sample extraction, extract preparation, and chromatography. Six laboratories used electron capture detectors, and four laboratories used mass spectrometry. Despite detailed written standardized procedures, there were large differences in the results reported by the participating laboratories. Total PCB concentration results were more precise than those of the individual PCB mixtures, and the measurement precisions were better for the electron capture determinations (relative standard deviation of 30 percent).

The approach of determining PCBs as various PCB formulations has several disadvantages. One of them is that the gas chromatographic patterns in environmental samples frequently are different from the corresponding Aroclor patterns because of possible degradation and irreversible adsorption of some PCBs in the environmental samples. Usually, PCBs enter the environment as mixtures, and, thus, their determination is complicated. Most important of all is that their determination as various Aroclor mixtures does not allow the determination of individual congeners. A procedure to measure concentrations of PCBs by using nine selected PCB congeners as concentration calibration standards was developed (2). Concentrations are measured for each isomer group with different levels of chlorination, and then the total PCB concentration is calculated. Software was also developed (3) to perform fully automated identification and measurement of PCBs in environmental samples. These automated procedures were capable of detecting more PCB congeners than the analyst could. For example, 52 congeners were detected and measured by automated procedures while only 42 were identified and measured by the analyst (3). The most important aspect of the automation in this case was the cost savings: for each data file, 12 to 15 hours were required by an experienced analyst to obtain the PCB data, whereas 0.5 hour of an analyst's time and less than one hour of computer file time were needed for automated data retrieval (3).

Another study, released recently by the EPA Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, deals with the determination of organochlorine pesticides and PCBs in water and soil samples by gas chromatography/mass spectometry (4). Either full-range or selected-ion monitoring is required; the choice of method depends on the pollutant levels to be determined. In the case of selected-ion monitoring, two analyses are necessary: one to detect the organochlorine pesticides and one to detect the PCBs. The PCBs are identified and measured as isomer groups distinguished by their level of chlorination. This procedure, identified as EPA Method 680 (4), is still being tested by EPA Cincinnati with real samples to obtain method precision and accuracy information.

A study worth mentioning which was not covered in the literature review summary presented in Appendix A is the validation of the Soxhlet extraction procedure (Method 3540). The objective of this study published by Michael, et al. (5), was to conduct a single-laboratory evaluation of the Soxhlet procedure detailed in Method 3540 for the determination of hazardous waste components in spent diatomaceous earth filter materials. The validation consisted of two phases: in Phase I, accuracy and precision data were obtained with diatomaceous earth samples fortified with various compounds including 4,4'-DDD and 4,4'-DDE; in Phase II, precision was determined by the analysis of diatomaceous earth samples fortified with a pesticide manufacturing waste. The results showed that Method 3540 is both accurate (average recovery ~90 percent) and precise (average percent relative standard deviation ~8 percent) when tested with diatomaceous earth samples.

SECTION 5

METHOD 8080 EVALUATION

Method 8080, as published in the Office of Solid Waste document entitled "Test Methods for Evaluating Solid Wastes," (6) provides sample extract cleanup and gas chromatographic conditions for the determination of part-per-billion (ppb) levels of organochlorine pesticides and PCBs in a variety of environmental samples including ground water, liquids, and solids. Following solvent extraction of the liquid samples using a separatory funnel (Method 3510) or continuous liquid-liquid extractor (Method 3520) and of the solid samples in a Soxhlet extractor (Method 3540) or with a sonicator (Method 3550), the extracts are cleaned up by Florisil chromatography. Elution of compounds from the Florisil column is performed with 6, 15, and 50 percent ethyl ether in hexane (200 mL each). All compounds listed in Method 8080, except six organochlorine pesticides, elute in Fraction I. From those six organochlorine pesticides, four (dieldrin, endosulfan II, endrin, and endrin aldehyde) elute in Fraction II, and two (endosulfan II and endosulfan sulfate) elute in Fraction III. Endrin aldehyde was also reported to appear in Fraction III (26 percent recovery).

There are no indications in the protocol of Method 8080 about possible overlapping of compounds between fractions and about the reproducibility of the elution scheme. Moreover, the fact that the organochlorine pesticides are not separated from the PCBs after Florisil cleanup is obviously an indication that such a scheme would be of no use for samples contaminated with both organochlorine pesticides and PCBs. Thus, the preliminary evaluation studies of Method 8080 described in this section were intended to: (a) determine the recoveries of the organochlorine pesticides and PCBs in the absence of matrix interferences, (b) determine the extent of overlapping of compounds between fractions, and (c) determine the efficiency of such a cleanup scheme with real samples.

Because of the complex nature of the PCB formulations, we have selected PCB-1016 and PCB-1260 for the evaluation studies. PCB-1016 is very similar to PCB-1242 in composition and contains about 40 percent chlorine. PCB-1260 contains about 60 percent chlorine. Thus, since we were mostly interested in the elution patterns of the PCBs and their recoveries after sample cleanup, we postulated that the use of the two PCBs will give us enough information about the procedure, while allowing us to quantify the PCBs in the samples by visual comparison of the sample extract chromatograms with PCB-1016 or PCB-1260 chromatograms.

5.1 EXPERIMENTAL

Column Chromatography Procedure

Florisil was dried overnight at 130°C and was calibrated by the lauric acid method (7). The amount of Florisil required per column was calculated to be 11.6 g. Separate column runs were performed in duplicate for standards of PCBs (PCB-1016 and PCB-1260), toxaphene, technical chlordane, pesticide group A (gamma-BHC, heptachlor, aldrin, heptachlor epoxide, endosulfan I, dieldrin, Kepone, endosulfan II, 4,4'-DDT, and endrin aldehyde) and pesticide group B (alpha-BHC, beta-BHC, delta-BHC, 4,4'-DDE, endrin, 4,4'-DDD, endosulfan sulfate, and 4,4'-methoxychlor). The amount of each compound added to the columns is listed in Table 2.

Hexane extracts of environmental samples and standards in 2 to 3 mL hexane were applied to the columns and eluted in 3 fractions: 200 mL 6-percent ethyl ether in hexane (Fraction I), 200 mL 15-percent ethyl ether in hexane (Fraction II), and 200 mL 50-percent ethyl ether in hexane (Fraction III). The fractions were collected separately in Kuderna-Danish flasks, and each fraction was concentrated to a 10-mL final volume in hexane. The fractions collected from pesticide group A were exchanged to 2-percent methanol in benzene because methanol has been found to enhance and stabilize the detector response of Kepone when Kepone is dissolved in nonpolar solvents (8).

Sample Analysis

Sample eluates from the Florisil fractionation were analyzed by gas chromatography using a ^{63}Ni electron capture detector. The chromatographic conditions are listed in Section 6.3 for the 1.5-percent OV-17/1.95-percent OV-210 column. Pesticide and PCB concentrations in the samples were determined by using external standard calibration.

5.2 RESULTS

The recoveries after Florisil cleanup are presented in Table 2 as percent recovered for each compound in each fraction, as well as the total percent recovered in the three fractions. The two sets of numbers for each fraction represent duplicate determinations. The spiking level is given as total µg loaded onto the Florisil column.

Overall, the recoveries are quantitative (except for Kepone), and the agreement between the duplicate experiments is excellent. However, the following discrepancies have been noted between this set of results and the recovery data given in Method 8080:

- Dieldrin did not elute in Fraction II only but was recovered in both Fractions I and II
- More Endosulfan I eluted in Fraction I than in Fraction II

TABLE 2. RESULTS OF THE FLORISIL EVALUATION STUDY

Compound	Spike level (μg)	Fraction I	Fraction II	Fraction III	Total
alpha-BHC	0.5	93.7; 92.5	9.7; 8.7		103 ; 101
beta-BHC	0.5	86.3; 82.9	10.5; 0		96.8; 82.9
gamma-BHC	0.5	75.7; 77.5	8.5; 8.1	1.5; <1.0	85.7; 85.6
delta-BHC	0.5	69.6; 60.5	30.6; 26.7		100 ; 87.2
Heptachlor	0.5	75.4; 77.6	5.3; 5.8		80.7; 83.4
Aldrin	0.5	77.8; 79.6	6.0; 6.6	2.8; 3.8	86.6; 90.0
Heptachlor epoxide	0.5	79.1; 80.1	15.1; 14.7		94.2; 94.8
Technical chlordane	5.0	93.1; 94.5	9.6; 7.6		103 ; 102
gamma-Chlordane[a]		89.4; 91.0	6.1; 6.1		95.5; 97.1
alpha-Chlordane[a]		97.8; 99.6	6.3; 6.3		104 ; 106
Endosulfan I	0.5	59.3; 64.2	29.0; 30.0		88.3; 94.2
4,4'-DDT	1.0	94.6; 95.0	8.7; 6.7		103 ; 102
Dieldrin	1.0	20.0; 24.1	71.5; 70.5		91.5; 94.6
Endrin	1.0	37.4; 42.8	63.6; 50.8		101 ; 93.6
Endosulfan II	1.0		60.0; 79.5	30.2; 14.6	90.2; 94.1
4,4'-DDD	1.0	93.4; 90.3	11.4; 10.3		105 ; 101
Endrin aldehyde	1.0		79.9; 90.0	10.6; 4.4	90.5; 94.4
Kepone	1.0		23.3; 25.3	22.6; 23.7	45.9; 49.0
Endosulfan sulfate	1.0		23.5; 23.4	57.4; 51.3	80.9; 74.7
4,4'-DDE	1.0	98.6; 93.4	9.6; 8.6		108 ; 102
4,4'-Methoxychlor	5.0	87.4; 83.9	28.9; 25.6		116 ; 109
Toxaphene	10.0	105 ; 108			105 ; 108
PCB-1016	10.0	86.3; 76.5	4.6; 4.4		90.9; 80.9
PCB-1260	10.0	91.5; 78.5	10.2; 9.0		102 ; 87.5

[a] Found in the technical chlordane mixture.

- A higher percentage of Endosulfan II eluted in Fraction II and not in Fraction III
- Endrin overlapped in Fractions I and II
- Endosulfan sulfate overlapped in Fractions II and III.

A possible explanation for the discrepancies might be the fact that the Florisil calcination temperature influences its adsorption properties, as has been reported (9). Difficulties in obtaining reproducible recoveries and overlapping of pesticides between fractions have also been reported (7,9,10).

Regardless of the reproducibility of the fractionation scheme, it appears that the Florisil fractionation is not suitable for those samples that are contaminated with both organochlorine pesticides and PCBs. Because PCBs are extracted and isolated along with the organochlorine pesticides, these two types of analytes need to be separated from each other to a larger extent to avoid cross-interference. To exemplify this on a real sample, we have extracted a liquid waste, identified as liquid waste No. 1, and spiked the extract with known amounts of the organochlorine pesticides and PCBs. Following fractionation of the extract on Florisil, the three fractions were analyzed by both packed and capillary column chromatography. Figures 1 through 4 show GC/EC chromatograms of the three fractions obtained from the unspiked and spiked liquid waste No. 1 extract. Because of the complexity of this matrix we were not able to determine any of the spiked compounds when the fractions were analyzed on the OV-17/OV-210 packed column. Compound resolution was significantly improved when the DB-5 capillary column was used; however, even then we were not able to find all of the spiking compounds. In view of these results, it does not appear that the Florisil fractionation should be considered for further evaluation. Subsequent sections of this report address the improvement of the methodology for the determination of the organochlorine pesticides and PCBs and the selection of a new cleanup procedure that will be further evaluated for incorporation in the Method 8080 protocol.

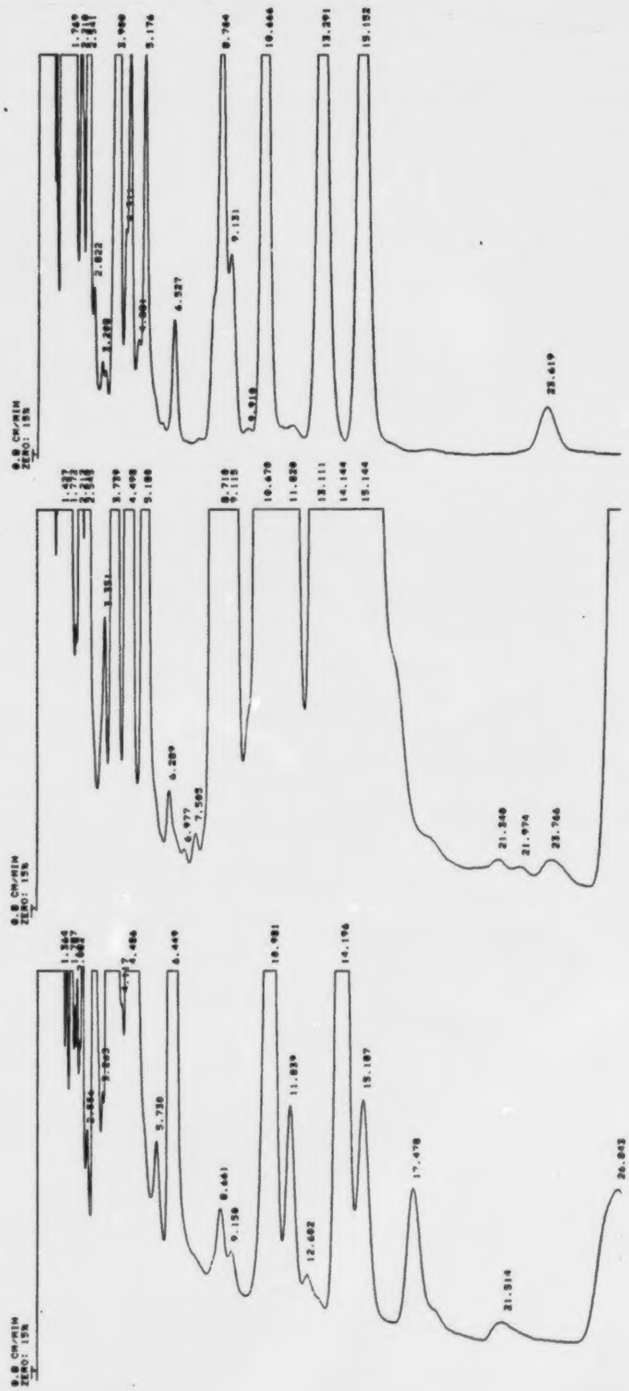

Figure 1. GE/EC chromatograms of the Florisil Fractions I (top), II (middle), and III (bottom) of liquid waste No. 1 extract, analyzed on the 1.8 m x 2 mm ID 1.5-percent OV-17/1.95-percent OV-210 column; isothermal at 190°C.

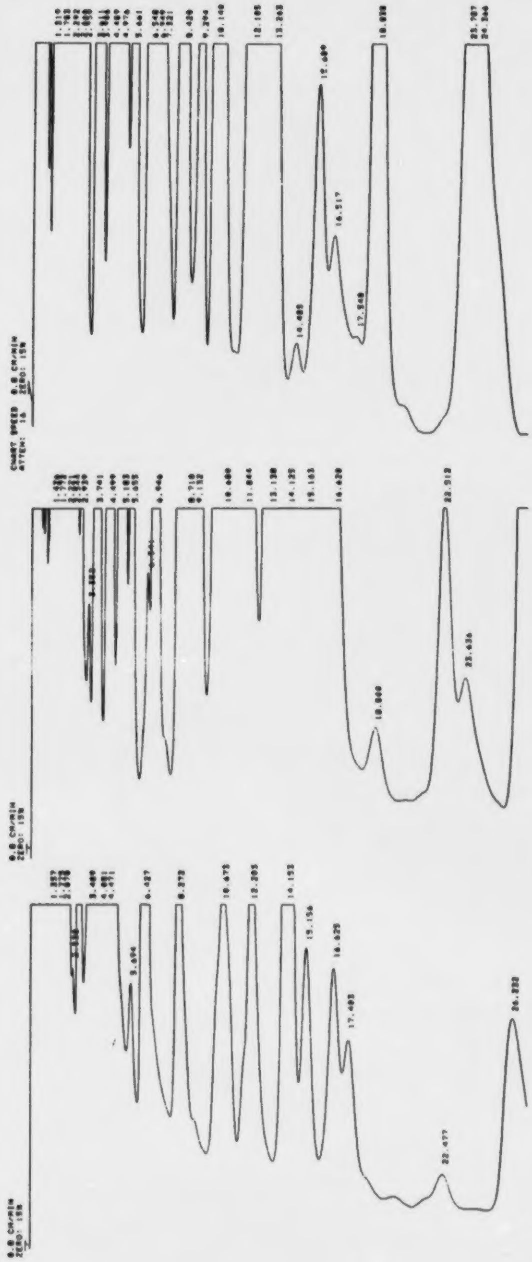

Figure 2. GE/EC chromatograms of the Florisil Fractions I (top), II (middle), and III (bottom) of liquid waste No. 1 extract, spiked with the organochlorine pesticides and PCBs, and analyzed on the 1.8 m x 2 mm ID 1.5-percent OV-17/1.95-percent OV-210 column; isothermal at 190°C.

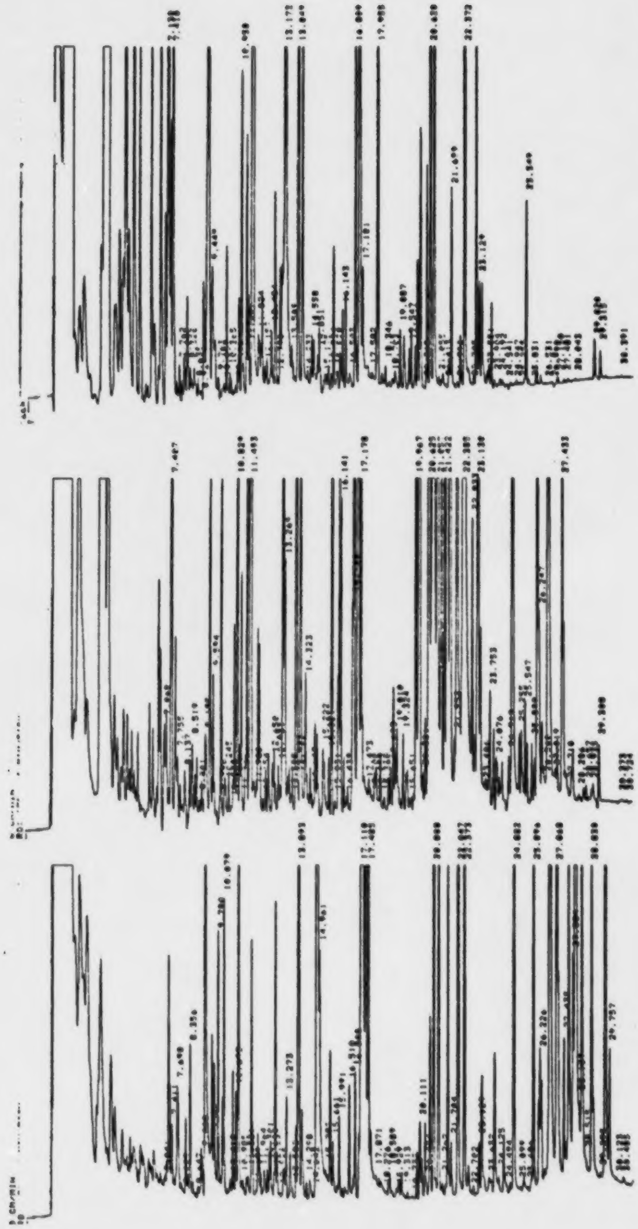

Figure 3. GE/EC chromatograms of the Florisil Fractions I (top), II (middle), and III (bottom) of liquid waste No. 1 extract, analyzed on the 30 m x 0.25 mm ID DB-5 fused-silica capillary column. Temperature program: 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; helium at 16 psi.

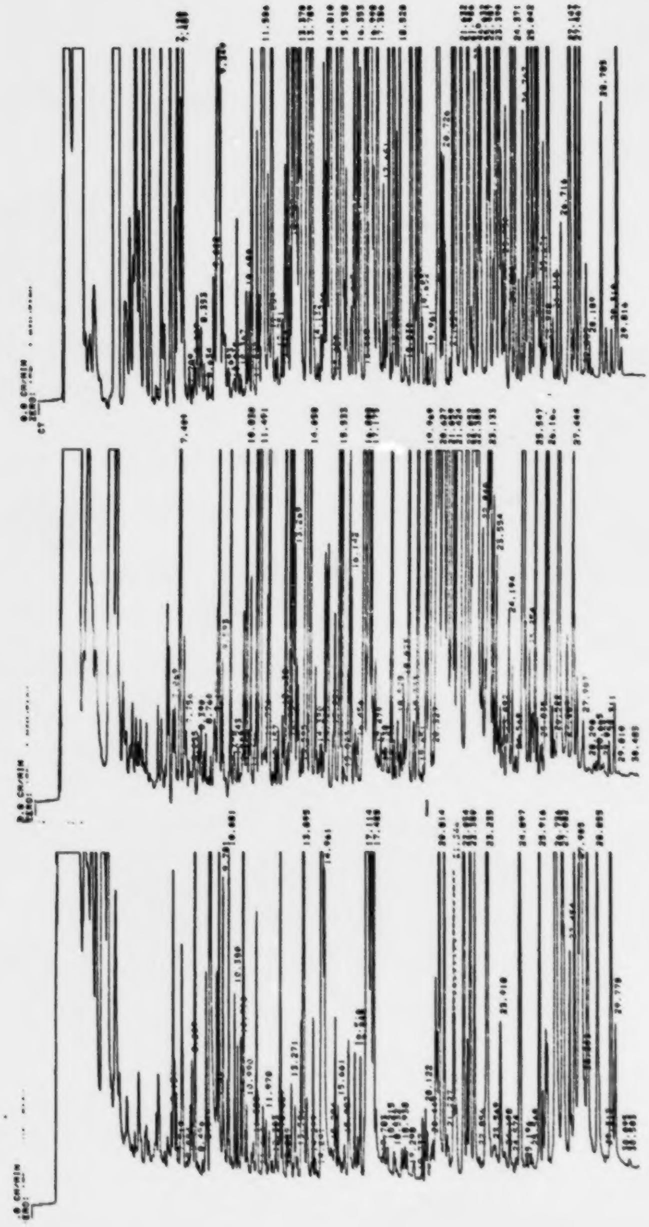

Figure 4. GC/EC chromatograms of the Florisil Fractions I (top), II (middle), and III (bottom) of liquid waste No. 1 extract, spiked with the organochlorine pesticides and PCBs, and analyzed on the 30 m x 0.25 mm ID DB-5 fused-silica capillary column. Temperature program: 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; helium at 16 psi.

SECTION 6

METHOD IMPROVEMENT

Improvement of the method for the determination of the organochlorine pesticides and PCBs in liquid and solid wastes focused on developing a fractionation procedure to separate the PCBs from the organochlorine pesticides and, at the same time, to remove interfering coextractants. Several extract cleanup procedures (e.g., silica, alumina, silica gel/Celite, Florisil/charcoal) were investigated, and a capillary gas chromatographic method was developed. Furthermore, we have investigated to a limited extent the extraction techniques recommended in the Method 8080 protocol. For example, we have used the four extraction techniques (Methods 3510, 3520, 3540, and 3550) to prepare the extracts that were used to evaluate cleanup techniques, and we have performed a ruggedness test for Method 3550 to determine how sensitive the method is to changes in solvent composition, sonicator output setting, volume of the extraction solvent, etc. The results of these experiments led to the development of a revised protocol for the analysis of the organochlorine pesticides and PCBs.

This section presents the results of the method improvement studies. Extraction will be addressed first and will be followed by cleanup techniques and gas chromatographic analysis.

6.1 EXTRACTION

The purpose of the extraction is to isolate quantitatively the compound(s) of interest from the matrix. Standard methodologies include extraction of liquid samples with an organic solvent using a separatory funnel or some sort of stirring device, whereas solid samples are extracted either in a Soxhlet extractor or with a homogenizer, tumbler, or sonicator. Different coextractants derived from the sample matrix may affect the determination of compounds of interest; therefore, sample extracts must usually be subjected to cleanup prior to analysis to reduce the amount of coextractants in the matrix. This section addresses the evaluation of four standard methodologies for sample extraction. These methodologies are identified as Methods 3510, 3520, 3540, and 3550 in SW-846.

6.1.1 Extraction of Aqueous Samples

Experimental

One-liter, distilled-water samples were spiked in triplicate at two concentrations (spike levels are listed in Tables 3 and 4), were extracted by

TABLE 3. EVALUATION OF METHOD 3510 USING DISTILLED WATER SAMPLES

	Spike level (µg/L)		Average Recovery ± SD (RSD)[a]	
Compound	Conc. 1	Conc. 2	Conc. 1	Conc. 2
alpha-BHC	0.5	5.0	94.2 ± 9.1 (9.7)	101 ± 2.8 (2.8)
beta-BHC	0.5	5.0	161[b] ± 17.5 (10.9)	187[b] ± 19.6 (10.5)
gamma-BHC	0.5	5.0	90.4 ± 8.9 (9.9)	98.8 ± 3.1 (3.2)
delta-BHC	0.5	5.0	90.0 ± 11.0 (12.2)	98.0 ± 5.3 (5.4)
Heptachlor	0.5	5.0	70.8 ± 7.9 (11.1)	85.5 ± 4.4 (5.2)
Aldrin	0.5	5.0	72.0 ± 12.9 (17.9)	88.9 ± 3.2 (3.5)
Heptachlor epoxide	0.5	5.0	78.6 ± 9.5 (12.1)	85.2 ± 3.8 (4.5)
gamma-Chlordane	1.0	10.0	73.5 ± 7.1 (9.7)	80.8 ± 4.1 (5.1)
Endosulfan I	0.5	5.0	86.9 ± 6.8 (7.9)	99.8 ± 6.6 (6.6)
4,4'-DDE + Dieldrin[c]	1.0	10.0	64.0 ± 7.4 (11.6)	74.6 ± 5.2 (7.0)
Endrin	1.0	10.0	98.0 ± 14.9 (15.2)	77.8 ± 7.6 (9.7)
Endosulfan II	1.0	10.0	63.0 ± 6.9 (10.9)	69.6 ± 7.0 (10.1)
4,4'-DDD	1.0	10.0	72.9 ± 8.3 (11.4)	77.6 ± 9.3 (12.0)
Endrin aldehyde	1.0	10.0	72.2 ± 8.4 (11.7)	86.6 ± 8.7 (10.0)
Kepone	1.0	10.0	58.5 ± 43.7 (75.0)	71.5 ± 27.3 (38.0)
Endosulfan sulfate	1.0	10.0	70.6 ± 8.5 (12.0)	77.3 ± 7.9 (10.3)
4,4'-DDT	1.0	10.0	84.8 ± 7.0 (8.2)	76.9 ± 9.7 (12.6)
4,4'-Methoxychlor	0.5	5.0	76.7 ± 10.1 (13.2)	74.1 ± 9.8 (13.2)
Toxaphene	10.0	100.0	93.6 ± 6.8 (7.3)	98.2 ± 11.9 (12.1)
PCB-1016	5.0	50.0	84.8 ± 6.8 (8.0)	87.5 ± 18.0 (20.6)
PCB-1260	5.0	50.0	91.9 ± 7.1 (7.7)	87.0 ± 15.3 (17.6)

SD -- Standard deviation.

RSD -- Relative standard deviation.

[a] Number of determinations is 3.

[b] High recovery possibly because of contamination of unknown origin.

[c] Spike level of 4,4'-DDE and dieldrin is 0.5 µg/L for conc. 1 and 5.0 µg/L for conc. 2 for each compound.

TABLE 4. EVALUATION OF METHOD 3520 USING DISTILLED WATER SAMPLES

	Spike level (µg/L)		Average Recovery ± SD (RSD)[a]	
Compound	Conc. 1	Conc. 2	Conc. 1	Conc. 2
alpha-BHC	0.5	5.0	102 ± 1.0 (1.0)	101 ± 2.5 (2.5)
beta-BHC	0.5	5.0	183[b] ± 3.2 (1.8)	173[b] ± 6.0 (3.5)
gamma-BHC	0.5	5.0	98.7 ± 1.2 (1.2)	98.6 ± 2.3 (2.3)
delta-BHC	0.5	5.0	101 ± 2.9 (2.9)	96.1 ± 2.4 (2.5)
Heptachlor	0.5	5.0	89.6 ± 3.7 (4.1)	85.2 ± 2.4 (2.9)
Aldrin	0.5	5.0	93.8 ± 1.8 (1.9)	90.5 ± 2.1 (2.4)
Heptachlor epoxide	0.5	5.0	87.6 ± 3.5 (4.0)	84.0 ± 2.1 (2.5)
gamma-Chlordane	1.0	10.0	82.9 ± 2.8 (3.3)	80.1 ± 1.9 (2.4)
Endosulfan I	0.5	5.0	96.6 ± 1.5 (1.6)	98.6 ± 2.5 (2.5)
4,4'-DDE + Dieldrin[c]	1.0	10.0	72.4 ± 1.4 (2.0)	71.4 ± 2.7 (3.7)
Endrin	1.0	10.0	118 ± 13.3 (11.3)	80.5 ± 4.7 (5.8)
Endosulfan II	1.0	10.0	72.8 ± 3.2 (4.4)	66.9 ± 1.3 (1.9)
4,4'-DDD	1.0	10.0	83.7 ± 5.4 (6.4)	74.4 ± 1.9 (2.6)
Endrin aldehyde	1.0	10.0	79.8 ± 6.0 (7.6)	84.6 ± 2.6 (3.1)
Kepone	1.0	10.0	69.8 ± 18.0 (25.9)	66.2 ± 8.3 (12.5)
Endosulfan sulfate	1.0	10.0	82.3 ± 6.9 (8.4)	76.0 ± 2.0 (2.6)
4,4'-DDT	1.0	10.0	102 ± 8.8 (8.6)	75.6 ± 2.0 (2.6)
4,4'-Methoxychlor	0.5	5.0	89.7 ± 14.4 (16.1)	72.9 ± 1.8 (2.4)
Toxaphene	10.0	100.0	74.4 ± 23.0 (31.0)	80.8 ± 14.8 (18.0)
PCB-1016	5.0	50.0	99.7 ± 4.8 (4.8)	97.9 ± 10.7 (10.9)
PCB-1260	5.0	50.0	91.0 ± 4.7 (5.2)	92.9 ± 5.1 (5.5)

SD -- Standard deviation.

RSD -- Relative standard deviation.

[a] Number of determinations is 3.

[b] High recovery possibly because of contamination of unknown origin.

[c] Spike level of 4,4'-DDE and dieldrin is 0.5 µg/L for conc. 1 and 5.0 µg/L for conc. 2 for each compound.

using the procedures given in Methods 3510 and 3520 (Appendix C), and the extracts were analyzed on a 30m x 0.25 mm ID DB-5 fused-silica capillary column (J&W Scientific, Rancho Cordova, CA).

Experiments were also performed with two liquid wastes identified as liquid wastes Nos. 1 and 2. Liquid waste No. 1 was collected from a pesticide waste storage facility at a California agricultural field station. This waste was found to contain high concentrations of organics (total organic carbon 520 mg/L; total organic halogen 30 mg/L). The following organochlorine pesticides were found in the sample (value given in parentheses is concentration in µg/L): alpha-BHC (0.39), beta-BHC (0.52), gamma-BHC (0.32), aldrin (0.05), heptachlor epoxide (0.11), gamma-chlordane (0.11), endosulfan I (0.51), 4,4'-DDE (0.53), endosulfan II (29), 4,4'-DDD (98), endrin aldehyde (220), and PCB-1260 (0.89). Liquid waste No. 2 was obtained from a pesticide manufacturing plant. The manufacturer reported the following: pH 7, NH3 as nitrogen 2200 mg/L, chemical oxygen demand 110,000 mg/L, chloride 21,000 mg/L, cyanide 8.6 mg/L, fluoride 330 mg/L, oil and grease 2400 mg/L, sulfate 12,000 mg/L, total dissolved solids 50,000 mg/L, potassium 1000 mg/L, sodium 16,000 mg/L, sodium acetate 1 percent, methanol 4.98 percent, acetone 0.67 percent, isopropyl alcohol 0.8 percent, tetrahydrofuran 1.98 percent, dimethylformamide 0.2 percent, methoprene 312 mg/L, and hydroprene 40 mg/L.

Results

The results of the Methods 3510 and 3520 evaluation studies are given in Tables 3 and 4. Only the results for the distilled water samples are presented since the extracts were not subjected to any cleanup procedure prior to the gas chromatographic analysis. The liquid wastes extracts were so complex that they had to be subjected to alumina cleanup prior to analysis. Consequently, any recovery data for spiked compounds in liquid wastes Nos. 1 and 2 had to be lower than those for the distilled water because of losses associated with alumina cleanup; they cannot be compared with those obtained for the distilled water samples.

The following conclusions can be drawn from the data in Tables 3 and 4:

- Methods 3510 and 3520 gave comparable accuracies when evaluated with spiked distilled water samples. Except for Kepone and endosulfan II, all compounds had recoveries >70 percent with either method.
- The precision was better for Method 3520. Thirty-five out of 42 RSD values for Method 3520 were below 8.6 percent, with most of these values below 5 percent. In the case of Method 3510, only 14 out of 42 RSD values were below 8.6 percent.

- In the case of liquid waste samples, it is difficult to conclude from the recovery data whether one method is better than the other, since cleanup was a must prior to the gas chromatographic analysis. In the case of liquid waste samples, the volume of sample to be extracted may be 100 mL, or less if the sample is highly contaminated. In that case, Method 3520 is not desirable since additional organics-free water has to be added to the sample for the continuous liquid-liquid extraction, and, subsequently, there is the possibility of introducing contamination.

6.1.2 Extraction of Soil Samples

Experimental

Sandy loam used in this study was obtained from Soils Incorporated, Puyallup, Washington. The following physico-chemical characteristics were determined: pH 5.9 to 6.0, 89 percent sand, 7 percent silt, 4 percent clay; cation exchange capacity 7 meq/100 g, and total organic carbon content 1290 ± 185 mg/kg.

The spiked sandy loam samples (spike levels are listed in Tables 5 and 6) were extracted by following the procedures detailed in Methods 3540 and 3550. Ten-gram portions of sandy loam soil spiked with the organochlorine pesticides (PCBs and toxaphene were spiked separately) were mixed with 10 g anhydrous Na_2SO_4, and the mixtures were then transferred to a Soxhlet extractor; 300 mL of hexane-acetone (41:59) was used for the 16-hour extraction. Sonication was also performed on 10-g portions of sandy loam soil. Water (3 mL) was added to each sample to wet the soil. The spike was added to the soil slurry and was allowed to equilibrate with the soil for 1 hour. Sonication was performed 3 times for 3 min with 60-mL portions of acetone-hexane (1:1). A sonicator Model W-375 from Heat Systems-Ultrasonics, Inc., in pulsed mode at 50 percent duty cycle, was employed. Sandy loam extracts were not subjected to any cleanup prior to gas chromatographic analysis. All analyses were performed on a 30 m x 0.25 mm ID DB-5 fused-silica capillary column (J&W Scientific, Rancho Cordova, CA).

Two other solid matrices, a National Bureau of Standards (NBS) reference sediment material (SRM 1645) and a solid matrix consisting of activated charcoal mixed with polymeric material, were also used in the evaluation studies. The NBS SRM 1645 has the following physico-chemical properties: Kjeldahl nitrogen, 0.0797 ± 0.26 percent; total phosphorus, 0.51 ± 0.0014 percent; loss on ignition (800°C), 10.72 ± 0.28 percent; oil and grease, 1.71 ± 0.26 percent; chemical oxygen demand, 149,400 ± 9,000 mg/Kg; SiO_2, 51 percent; MgO, 4 percent; Al_2O_3, 4 percent; CaO, 4 percent; total organic carbon, 30,000 mg/Kg. A physico-chemical characterization of the charcoal/polymeric material matrix was not performed. Extraction of the NBS SRM 1645 and of the solid matrix were performed as described above for the sandy loam.

TABLE 5. EVALUATION OF METHOD 3540 USING SANDY LOAM SAMPLES

	Spike level (mg/Kg)		Average Recovery ± SD (RSD)[a]	
Compound	Conc. 1	Conc. 2	Conc. 1	Conc. 2
alpha-BHC	0.05	0.5	50.4 ± 29.2 (59)	53.0 ± 33.8 (64)
beta-BHC	0.05	0.5	84.8 ± 6.2 (7.3)	115 ± 71.4 (62)
gamma-BHC	0.05	0.5	52.4 ± 27.7 (53)	55.8 ± 33.6 (60)
delta-BHC	0.05	0.5	34.0 ± 35.7 (105)	43.6 ± 43.7 (100)
Heptachlor	0.05	0.5	46.0 ± 23.4 (51)	46.5 ± 23.8 (51)
Aldrin	0.05	0.5	47.5 ± 22.9 (48)	49.4 ± 23.1 (47)
Heptachlor epoxide	0.05	0.5	61.5 ± 28.5 (46)	59.5 ± 21.2 (36)
gamma-Chlordane	0.10	1.0	52.8 ± 13.5 (26)	56.6 ± 20.2 (36)
Endosulfan I	0.05	0.5	51.8 ± 14.6 (28)	64.2 ± 10.5 (16)
4,4'-DDE + Dieldrin[c]	0.10	1.0	67.9 ± 10.0 (30)	60.7 ± 12.9 (21)
Endrin	0.10	1.0	92.1 ± 37.5 (41)	77.2 ± 13.6 (18)
Endosulfan II	0.10	1.0	45.5 ± 22.1 (49)	50.8 ± 6.2 (12)
4,4'-DDD	0.10	1.0	76.2 ± 12.5 (17)	74.8 ± 5.7 (7.7)
Endrin aldehyde	0.10	1.0	16.2 ± 7.6 (47)	22.5 ± 6.1 (27)
Kepone	0.10	1.0	17.1 ± 16.2 (95)	26.2[b]
Endosulfan sulfate	0.10	1.0	46.9 ± 31.6 (68)	50.6 ± 15.1 (30)
4,4'-DDT	0.10	1.0	66.7 ± 31.4 (47)	63.3 ± 7.8 (12)
4,4'-Methoxychlor	0.50	5.0	75.9 ± 11.3 (15)	70.3 ± 6.9 (9.9)
Toxaphene	1.0	1.0	35.0 ± 10.5 (30)	66.1 ± 19.7 (30)
PCB-1016	0.50	5.0	70.9 ± 26.5 (37)	59.4 ± 23.2 (39)
PCB-1260	0.50	5.0	71.8 ± 16.7 (23)	86.9 ± 14.9 (17)

SD -- Standard deviation.
RSD -- Relative standard deviation.

[a] Number of determinations is 3.

[b] Kepone recovered only in one replicate.

[c] Spike level of 4,4'-DDE and dieldrin is 0.05 mg/Kg for conc. 1 and 0.5 mg/Kg for conc. 2 for each compound.

TABLE 6. EVALUATION OF METHOD 3550 USING SANDY LOAM SAMPLES

Compound	Spike level (mg/Kg)		Average Recovery ± SD (RSD)[a]	
	Conc. 1	Conc. 2	Conc. 1	Conc. 2
alpha-BHC	0.05	0.5	73.4 ± 6.8 (9.2)	80.4 ± 5.5 (6.8)
beta-BHC	0.05	0.5	139[b] ± 5.6 (4.0)	150[b] ± 9.2 (6.2)
gamma-BHC	0.05	0.5	72.1 ± 5.0 (6.9)	82.1 ± 2.1 (2.5)
delta-BHC	0.05	0.5	59.1 ± 34.1 (58)	82.3 ± 2.6 (3.2)
Heptachlor	0.05	0.5	71.2 ± 2.4 (3.4)	68.4 ± 4.7 (6.9)
Aldrin	0.05	0.5	73.2 ± 2.2 (3.0)	73.2 ± 5.1 (7.0)
Heptachlor epoxide	0.05	0.5	72.8 ± 3.1 (4.2)	69.7 ± 4.7 (6.8)
gamma-Chlordane	0.10	1.0	66.4 ± 1.2 (1.7)	66.1 ± 4.1 (6.3)
Endosulfan I	0.05	0.5	58.7 ± 0.6 (1.0)	69.5 ± 4.6 (6.6)
4,4'-DDE + Dieldrin[c]	0.10	1.0	75.4 ± 1.3 (1.7)	63.7 ± 3.6 (5.6)
Endrin	0.10	1.0	95.3 ± 5.3 (5.6)	69.0 ± 4.3 (6.2)
Endosulfan II	0.10	1.0	48.8 ± 2.2 (4.5)	51.1 ± 3.0 (5.9)
4,4'-DDD	0.10	1.0	71.2 ± 3.3 (4.6)	63.8 ± 3.7 (5.8)
Endrin aldehyde	0.10	1.0	89.4 ± 58.8 (66)	29.7 ± 4.2 (14.2)
Kepone	0.10	1.0	18.3 ± 23.2 (12.6)	23.9 ± 4.4 (18.3)
Endosulfan sulfate	0.10	1.0	61.5 ± 4.9 (7.9)	60.2 ± 4.0 (6.6)
4,4'-DDT	0.10	1.0	66.4 ± 4.7 (7.1)	58.1 ± 4.4 (7.5)
4,4'-Methoxychlor	0.50	5.0	73.0 ± 4.9 (6.7)	58.6 ± 3.2 (5.5)
Toxaphene	1.0	1.0	48.1 ± 16.8 (35)	77.3 ± 18.5 (24)
PCB-1016	0.50	5.0	72.1 ± 6.8 (9.4)	89.4 ± 11.9 (13.3)
PCB-1260	0.50	5.0	83.3 ± 4.3 (5.2)	80.6 ± 7.9 (9.8)

SD -- Standard deviation.

RSD -- Relative standard deviation.

[a] Number of determinations is 3.

[b] High recovery possibly because of interference from matrix.

[c] Spike level of 4,4'-DDE and dieldrin is 0.05 mg/Kg for conc. 1 and 0.5 mg/Kg for conc. 2 for each compound.

Spiking the NBS sediment and the solid matrix with the substances of interest was performed by adding 3 and 5 mL methanol, respectively, to wet the solids and to allow for better mixing of the spike with the solid. In each case, the solid material was allowed to dry at room temperature for approximately 30 min prior to extraction. All extracts except those from sandy loam were cleaned up by using alumina deactivated by 15 percent water.

Results

The results of the Methods 3540 and 3550 evaluation studies are given in Tables 5 and 6. Only the results for the sandy loam samples are presented since the extracts were not subjected to any cleanup prior to the gas chromatographic analysis. The NBS sediment and solid material extracts were subjected to alumina cleanup prior to analysis. Consequently, any recovery data for the spiked compounds in the NBS sediment and the solid wastes were lower than those obtained for the sandy loam because of losses associated with alumina cleanup, and they cannot be compared with the recovery data obtained for the sandy loam samples.

The following conclusions can be drawn from the data presented in Tables 5 and 6:

- Method 3550 appears to give better precision than Method 3540. Thirty-four out of 42 RSD values for Method 3550 were below 9.8 percent. Most of the RSD values for Method 3540 were in the 30- to 40-percent range.
- The accuracies of both methods were similar. Low recoveries (~20 percent) were found for Kepone by either method.
- Spiking of the matrix with the test compounds may have contributed to the variability of the extraction recoveries. In the case of Method 3540, separate sample portions were spiked in Petri dishes and then were transferred to the Soxhlet extractor. Water was added to the sandy loam samples to slurry and to distribute the spike. Methanol was added to the sediment reference material since water did not seem to wet the sediment. Spike volatilization from the open Petri dish and some loss during the transfer of the solid material to the Soxhlet extractor cannot be ruled out. In the case of Method 3550, the solid material portions were spiked when they were already in the extraction jar; thus, losses associated with sample transfer were probably small.
- The determination of the compounds is affected by interferences from the matrix. In the case of sandy loam which gave relatively clean extracts, all compounds could be determined by either method; Method 3550 gave better precision than Method 3540. With the standard reference material and the solid matrix, matrix interferences precluded the identification of 4,4'-DDT, toxaphene, endosulfan I, dieldrin, 4,4'-DDE, endrin, endosulfan II, and gamma-chlordane even after alumina cleanup.

6.1.3 Ruggedness Test for Method 3550

A ruggedness test was performed for Method 3550 to determine how sensitive the method is to changes of the parameters listed in Table 7: extraction solvent composition, sonicator output setting, solvent volume, moisture content, analyte concentration, extract filtration, and the number of times the extraction was repeated. The seven parameters are assigned the letters A through G, and the changed values are assigned the same letters in the lower case. For example, the extraction solvent is hexane-acetone (1:1) in experiments 1 through 4 and hexane-acetone (3:1) in experiments 5 through 8.

TABLE 7. COMBINATIONS OF 7 PARAMETERS RECOMMENDED FOR TESTING THE RUGGEDNESS OF METHOD 3550

Condition	Experiment no. 1	2	3	4	5	6	7	8
Extraction solvent composition (hexane-acetone 1:1; 3:1)	A	A	A	A	a	a	a	a
Sonicator output setting (8; 9)	B	B	b	b	B	B	b	b
Solvent volume (60 mL, 120 mL)	C	c	C	c	C	c	C	c
Volume of water added to soil (0; 3 mL)	D	D	d	d	d	d	D	D
Analyte concentration (10 ppb; 250 ppb)	E	e	E	e	e	E	e	E
Extract filtration (Celite; Whatman filter)	F	f	f	F	F	f	f	F
Number of times the extraction was repeated (1; 3)	G	g	g	G	g	G	G	g
Result (percent recovery of each test compound)	s	t	u	v	w	x	y	z

The analytical results are presented as percent recovery for each organochlorine pesticide in each of the eight experiments (Table 8). Table 9 shows the group differences VA through VG which were calculated from equations 1 through 7:

$$VA = 1/4(s + t + u + v) - 1/4(w + x + y + z) = A-a \quad (1)$$
$$VB = 1/4(s + t + w + x) - 1/4(u + v + y + z) = B-b \quad (2)$$
$$VC = 1/4(s + u + w + y) - 1/4(t + v + x + z) = C-c \quad (3)$$
$$VD = 1/4(s + t + y + z) - 1/4(u + v + w + x) = D-d \quad (4)$$
$$VE = 1/4(s + u + x + z) - 1/4(t + v + w + y) = E-e \quad (5)$$
$$VF = 1/4(s + v + w + z) - 1/4(t + u + x + y) = F-f \quad (6)$$
$$VG = 1/4(s + v + x + y) - 1/4(t + u + w + z) = G-g \quad (7)$$

For example, VA for alpha-BHC represents the average for the A determinations minus the average for the a determinations [e.g., VA = 1/4(82 + 80 + 86 +68) -1/4(85 + 77 + 82 + 93) = -5.25], which means that determinations when hexane-acetone (1:1) was used as extraction solvent gave lower average recoveries than the determinations when hexane-acetone (3:1) was used as the extraction solvent. Furthermore, for alpha-BHC, parameters such as the number of times the extraction was repeated, the analyte concentration, the hexane-to-acetone ratio, and the moisture content of the soil, stand out in Table 9 as having greater effects on the analytical results than the other three parameters. No attempt was made to determine the significance of these four large differences, only those parameters were identified that may be considered for further investigation.

The following is a brief summary of the parameters which seem to affect the performance of the method:

- The change in the composition of the solvent was found to affect the performance of the method for almost all compounds; it is interesting to note that the algebraic signs of the differences in Table 9, Column 1, are mostly negative. This indicates that for most compounds extraction with hexane-acetone (3:1) gave higher recoveries.
- Sonicator output setting, solvent volume, and extract filtration did not appear to affect the performance of the method.
- As expected, improved recoveries were obtained when the extraction was repeated three times.
- Analyte concentration appeared to have a strong effect upon compound recovery; this was quite evident for dieldrin and 4,4'-DDE for which V_E was found to be 50.75, and for endrin aldehyde for which V_E was found to be -10.75. Gas chromatographic analysis probably plays a major role in both cases since in the case of dieldrin and 4,4'-DDE the detector response was slightly saturated when the extract from the 250 ppb soil was analyzed, whereas for endrin aldehyde the response was not linear at lower concentrations because of the possible degradation of endrin which was also present in the sample extract.

TABLE 8. RUGGEDNESS TEST FOR METHOD 3550 -- RECOVERY DATA FOR THE ORGANOCHLORINE PESTICIDES

	Percent recovery							
Experiment	1 (s)	2 (t)	3 (u)	4 (v)	5 (w)	6 (x)	7 (y)	8 (z)
Compound								
alpha-BHC	82	80	86	68	85	77	82	93
beta-BHC	73	80	72	64	82	62	81	81
gamma-BHC	NA	91	NA	103	95	NA	89	NA
delta-BHC	79	75	82	66	86	83	84	101
Heptachlor	132	82	97	72	88	86	84	NA
Aldrin	88	82	86	67	85	73	84	101
Heptachlor epoxide	77	80	81	63	84	73	84	90
gamma-Chlordane	95	81	79	64	85	72	83	92
Endosulfan I	112	77	76	65	75	83	76	88
4,4'-DDE + Dieldrin	110	47	106	37	49	95	95	120
Endrin	105	86	100	73	112	110	101	145
Endosulfan II	99	79	78	66	87	77	86	93
4,4'-DDD	99	81	89	66	89	87	86	106
Endrin aldehyde	53	65	61	67	71	58	76	64
Endosulfan sulfate	NA	80	70	67	87	74	85	98
4,4'-DDT	NA	87	112	67	93	137	88	158
4,4'-Methoxychlor	121	83	107	74	92	112	88	139

NA -- Not able to determine recovery because of interference.

Based on the results of the ruggedness test, it seemed that additional work would be required to determine how the extraction recoveries vary with the change in the composition of the extraction solvent. Therefore, other experiments were performed in which sandy loam soil spiked with the organochlorine pesticides was extracted with hexane/acetone (60 mL) mixtures using sonication (sonicator output setting: 8, sonicator time: 3 min). The extraction was performed with hexane/acetone 1:1, 2:1, 3:1, and hexane only, and was repeated three times in each instance. The extracts were filtered through a Whatman filter. The analyte concentration was kept constant at 250 ppb for each compound.

TABLE 9. RUGGEDNESS TEST -- GROUP DIFFERENCES FOR THE ORGANOCHLORINE PESTICIDES

Compound	V_A	V_B	V_C	V_D	V_E	V_F	V_G
alpha-BHC	-5.25	-1.25	4.25	5.25	5.75	0.75	-8.75
beta-BHC	-4.25	0	5.25	8.75	-4.75	1.25	-8.75
gamma-BHC	a	a	a	a	a	a	a
delta-BHC	-13.0	-2.50	1.50	5.50	8.50	2.00	-8.00
Heptachlor	a	a	a	a	a	a	a
Aldrin	-5.00	-2.50	5.00	11.00	7.50	4.00	-10.5
Heptachlor epoxide	-7.50	-1.00	5.00	7.50	2.50	-1.00	-9.50
gamma-Chlordane	-3.25	3.75	8.25	12.75	6.25	5.25	-5.75
Endosulfan I	2.00	10.50	6.50	13.50	16.50	7.00	5.00
4,4'-DDE + Dieldrin	-14.75	-14.25	15.25	21.25	50.75	-6.75	3.75
Endrin	-26	-1.50	1.00	10.50	22.00	9.50	-13.5
Endosulfan II	-5.25	4.75	8.75	12.25	7.25	6.25	-2.25
4,4'-DDD	-8.25	2.25	5.75	10.25	14.75	4.25	-6.75
Endrin aldehyde	-5.75	-5.25	1.75	0.25	-10.75	-1.25	-1.75
Endosulfan sulfate	a	a	a	a	a	a	a
4,4'-DDT	a	a	a	a	a	a	a
4,4'-Methoxychlor	-11.50	0	0	11.50	35.50	9.00	-6.50

[a]Not able to calculate because some recovery values are missing.

The results are presented in Table 10. It can be concluded that overall the recoveries were slightly higher when hexane was used as the extraction solvent, however, the increase was insignificant and does not warrant a change in the Method 3550 protocol.

6.2 EXTRACT CLEANUP

6.2.1 Silica Gel Cleanup

The silica gel fractionation was performed as specified in a procedure developed by Biddleman, et al. (11), with slight modifications. The silica gel (Davidson 923, 100/200 mesh, Supelco, Inc.) was dried for at least 16 hours at 130 to 140°C. Three-g portions were weighed into vials, were redried for 1 hour, and were deactivated with 0.10 mL of water. The contents of each vial were mixed thoroughly and were equilibrated for an additional hour; afterwards, they were emptied into 11-mm-ID glass columns and were topped with 2 to 3 cm of sodium sulfate. Twenty mL dichloromethane followed by 20 mL hexane were used to wash the column.

TABLE 10. RUGGEDNESS TEST -- PERCENT RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AS A FUNCTION OF SOLVENT COMPOSITION

Compound	Percent Recovery			
	Hexane-Acetone 1:1	Hexane-Acetone 2:1	Hexane-Acetone 3:1	Hexane Only
alpha-BHC	75.2	64.3	79.7	87.1
beta-BHC	74.2	69.2	75.8	76.6
gamma-BHC	75.3	70.4	78.2	85.0
delta-BHC	73.0	67.2	76.9	78.5
Heptachlor	76.8	53.6	79.2	86.7
Aldrin	77.2	53.5	75.0	84.7
Heptachlor epoxide	74.0	63.9	75.0	80.8
gamma-Chlordane	73.8	62.1	75.2	82.5
Endosulfan I	73.1	60.1	72.0	80.2
4,4'-DDE + Dieldrin	82.8	70.0	81.2	82.8
Endrin	94.5	72.7	90.3	88.4
Endosulfan II	73.3	60.1	73.3	76.4
4,4'-DDD	75.2	61.9	74.2	77.1
Endrin aldehyde	66.6	43.5	56.1	65.9
Endosulfan sulfate	73.2	56.5	71.0	71.9
4,4'-DDT	79.5	53.1	73.0	75.0
4,4'-Methoxychlor	89.6	60.9	78.9	80.3
Average ± SD (%RSD)	76.9 ± 6.7 (8.7)	61.4 ± 7.6 (12.4)	75.6 ± 6.7 (8.9)	80.0 ± 5.9 (7.3)

SD -- Standard deviation.
RSD -- Relative standard deviation.

Separate experiments were run in triplicate and at two concentrations for each set of organochlorine pesticides and PCBs mixed standards. Technical chlordane and toxaphene standards were run separately. Standards of the individual pesticides (alpha-BHC, beta-BHC, gamma-BHC, delta-BHC, heptachlor, aldrin, heptachlor epoxide, endosulfan I, dieldrin, 4,4'-DDE, Kepone, endrin, endosulfan 11, 4,4'-DDD, 4,4'-DDT, endrin aldehyde, endosulfan sulfate, and 4,4'-methoxychlor) and PCB-1016 and PCB-1260 were run together. The amount of each compound added to the columns is given in Table 11.

The spikes were added to the column in 3 to 4 mL of hexane and were eluted in four fractions with 80 mL hexane (Fraction I), an additional 50 mL hexane (Fraction II), 15 mL methylene chloride (Fraction III), and 50 mL ethyl acetate (Fraction IV). The fractions were collected separately in Kuderna-Danish flasks and were concentrated to a 10-mL final volume in hexane. Because methanol has been found to enhance and stabilize the response of Kepone, the ethyl acetate (Fraction IV) was exchanged to 2 percent methanol in benzene.

Six extracts from distilled water, liquid waste No. 1, liquid waste No. 2, sandy loam, NBS sediment, and solid waste were also subjected to the silica gel cleanup. Two-milliliter aliquots from each extract were spiked with 500 or 5,000 ng of each organochlorine pesticide and with 5,000 or 50,000 ng of PCB-1016 and PCB-1260. Technical chlordane was replaced by gamma-chlordane; it was part of the organochlorine pesticides spike. Kepone was not spiked at all; however, we did collect and analyze Fraction IV of each extract to verify that none of the organochlorine pesticides eluted in Fraction IV. Toxaphene was not used since toxaphene does not elute from the silica gel column in a narrow band as do the other compounds, and it may preclude the determination of the organochlorine pesticides and PCBs.

Tables 12 through 19 present the data for the 6 matrix extracts spiked with the organochlorine pesticides and PCB-1016 and PCB-1260. For each matrix, unspiked extracts were also processed through the silica gel column to determine which of the organochlorine pesticides and PCBs listed in Method 8080 was present in the unspiked sample extracts.

The following conclusions can be drawn from these data:

- The distribution patterns of the organochlorine pesticides and PCBs in the four fractions were quite reproducible, with a few exceptions (i.e., liquid waste No. 2). Compounds found to elute in Fraction I include heptachlor, aldrin, 4,4'-DDE, 4,4'-DDT, and PCBs. Almost all the other organochlorine pesticides elute in Fraction III. Only a few compounds (4,4'-DDD, alpha-BHC, gamma-chlordane) were found in Fraction II. The advantage of the four-fraction scheme over the Florisil scheme is in the separation of the PCBs from most of the organochlorine pesticides.
- All compounds were quantitatively recovered from the silica gel column. Total recoveries, listed in Tables 11 through 19, were greater than 70 percent, with most values ranging from 80 to 110 percent. Exceptions were Kepone and technical chlordane when they were spiked at concentration 1 (Table 11).

TABLE 11. DISTRIBUTION AND PERCENT RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs IN THE SILICA GEL COLUMN FRACTIONS[a,b,c,d,e]

Compound	Fraction I		Fraction II		Fraction III		Fraction IV		Total recovery	
	Conc. 1	Conc. 2	Conc. 1	Conc. 2	Conc. 1	Conc. 2	Conc. 1	Conc. 2	Conc. 1	Conc. 2
alpha-BHC					82 (1.7)	74 (8.0)			82 (1.7)	74 (8.0)
beta-BHC					107 (2.1)	98 (12.5)			107 (2.1)	98 (12.5)
gamma-BHC					91 (3.6)	85 (10.7)			91 (3.6)	85 (10.7)
delta-BHC					92 (3.5)	83 (10.6)			92 (3.5)	83 (10.6)
Heptachlor	109 (4.1)	118 (8.7)							109 (4.1)	118 (8.7)
Aldrin	97 (5.6)	104 (1.6)							97 (5.6)	104 (1.6)
Heptachlor epoxide					95 (4.7)	88 (10.2)			95 (4.7)	88 (10.2)
Endosulfan I					95 (5.1)	87 (10.2)			95 (5.1)	87 (10.2)
4,4'-DDE	86 (5.4)	94 (2.8)							86 (5.4)	94 (2.8)
Dieldrin					96 (6.0)	87 (10.6)			96 (6.0)	87 (10.6)
Endrin					85 (10.5)	71 (12.3)			85 (10.5)	71 (12.3)
Endosulfan II					97 (4.4)	86 (10.4)			97 (4.4)	86 (10.4)
4,4'-DDD					102 (4.6)	92 (10.2)			102 (4.6)	92 (10.2)
Endrin aldehyde					81 (1.9)	76 (9.5)	29 (4.2)	33 (4.7)	110 (3.0)	109 (5.8)
Kepone							41 (7.9)	73 (12.0)	41 (7.9)	73 (12.0)
Endosulfan sulfate					93 (4.9)	82 (9.2)			93 (4.9)	82 (9.2)
4,4'-DDT			86 (13.4)	73 (9.1)	15 (18.7)	8.7 (15.0)			101 (5.3)	82 (23.7)
4,4'-Methoxychlor					99 (9.9)	82 (10.7)	9.0 (4.8)	5.7 (3.0)	108 (10.0)	88 (5.8)
PCB-1016	86 (4.0)	87 (6.1)							86 (4.0)	87 (6.1)
PCB-1260	91 (4.1)	95 (5.0)							91 (4.1)	95 (5.0)
Technical chlordane	14 (5.5)	22 (5.3)	19 (6.8)	39 (3.6)	29 (5.0)	37 (5.1)			62 (3.3)	98 (1.9)
Toxaphene			15 (2.4)	17 (1.4)	73 (9.4)	84 (10.7)			88 (12.0)	101 (10.1)

[a] Eluant composition:
Fraction I - 80 mL hexane.
Fraction II - 50 mL hexane.
Fraction III - 15 mL methylene chloride.
Fraction IV - 50 mL ethyl acetate.

[b] Concentration 1 is 0.5 μg per column for BHCs, heptachlor, aldrin, heptachlor epoxide, endosulfan I; 1.0 μg per column for dieldrin, Kepone, endosulfan II, 4,4'-DDT, endrin aldehyde, 4,4'-DDD, 4,4'-DDE, endrin, and endosulfan sulfate; 5 μg per column for 4,4'-methoxychlor and technical chlordane; 10 μg per column for toxaphene, PCB-1016,and PCB-1260.

[c] For concentration 2 the amounts spiked are 10 times as high as those for concentration 1.

[d] The values given represent the average recoveries of three determinations; the numbers in parentheses are the standard deviations; recovery cutoff point is 5 percent.

[e] Data obtained with standards, as indicated in footnotes b and c, dissolved in 2 mL hexane.

TABLE 12. DISTRIBUTION AND PERCENT RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs SPIKED INTO THE DISTILLED WATER EXTRACTS (SPIKE LEVEL IS 500 NG FOR PESTICIDES AND 5,000 NG FOR PCBs)

	Percent recovery				
Compounds	Fraction I	Fraction II	Fraction III	Fraction IV	Total
alpha-BHC		36	56		92
beta-BHC			79		79
gamma-BHC			95		95
delta-BHC			83		83
Heptachlor	100				100
Aldrin	88				88
Heptachlor epoxide			85		85
gamma-Chlordane	60	39			99
Endosulfan I			83		83
4,4'-DDE	112				112
Dieldrin			97		97
Endrin			20		20
Endosulfan II			83		83
4,4'-DDD		17	70		87
Endrin aldehyde			87	9.8	97
Kepone				NS	NS
Endosulfan sulfate			82	16	98
4,4'-DDT	92				92
4,4'-Methoxychlor			71		71
PCB-1016 PCB-1260	97				97

NS -- Not spiked.

- Although total recoveries were quantitative for all compounds spiked into liquid waste No. 2 followed by fractionation on silica gel, the distribution patterns of the compounds were affected by the fact that the extract was applied to the column in benzene (because of solubility problems) and not in hexane.
- From the examination of the GC/EC chromatograms it is evident that even for very complex matrices such as liquid waste No. 1 (Figures 5 and 6), liquid waste No. 2, and the NBS sediment material, the fractionation of the extract on the silica gel column allows precise determination of the organochlorine pesticides and PCBs.

TABLE 13. DISTRIBUTION AND PERCENT RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs SPIKED INTO THE LIQUID WASTE NO. 1 EXTRACT (SPIKE LEVEL IS 500 NG FOR PESTICIDES AND 5,000 NG FOR PCBs)

Compounds	Percent recovery				
	Fraction I	Fraction II	Fraction III	Fraction IV	Total
alpha-BHC		41	76		117
beta-BHC			134		134
gamma-BHC			64		64
delta-BHC			218[a]		218[a]
Heptachlor	121				121
Aldrin	123				123
Heptachlor epoxide			135		135
gamma-Chlordane	78	57			135
Endosulfan I			118		118
4,4'-DDE	NA				NA
Dieldrin			160[a]		160[a]
Endrin			NA		NA
Endosulfan II			121		121
4,4'-DDD	NA				NA
Endrin aldehyde			NA		NA
Kepone				NS	NS
Endosulfan sulfate			NA		NA
4,4'-DDT	131				131
4,4'-Methoxychlor			118		118
PCB-1016	95				95
PCB-1260					

NA -- Not able to quantify because of interference.
NS -- Not spiked.

[a] High recovery probably because of matrix interference.

- Careful control of adsorbent activity (i.e., water content) is essential for reproducible separations. The silica gel material should be tested prior to use by using standards at concentrations equivalent to those expected to be found in the samples.

TABLE 14. DISTRIBUTION AND PERCENT RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs SPIKED INTO THE LIQUID WASTE NO. 1 EXTRACT (SPIKE LEVEL IS 5,000 NG FOR PESTICIDES AND 50,000 NG FOR PCBs)

	Percent recovery				
Compounds	Fraction I	Fraction II	Fraction III	Fraction IV	Total
alpha-BHC		36	55		91
beta-BHC			89		89
gamma-BHC			59		59
delta-BHC			NA		NA
Heptachlor	97				97
Aldrin	85				85
Heptachlor epoxide			86		86
gamma-Chlordane	65	24			89
Endosulfan I			88		88
4,4'-DDE	113				113
Dieldrin			110		110
Endrin			76		76
Endosulfan II			163[a]		163[a]
4,4'-DDD		11	74		85
Endrin aldehyde			80		80
Kepone				NS	NS
Endosulfan sulfate			156[a]		156[a]
4,4'-DDT	79				79
4,4'-Methoxychlor			140[a]		140[a]
PCB-1016 PCB-1260	84				84

NA -- Not able to quantify due to interference.
NS -- Not spiked.

[a] High recovery probably due to matrix interference.

Interference from phthalate esters was very briefly assessed using diethylphthalate, di-*n*-butylphthalate and di-*n*-octylphthalate. Five micrograms of each phthalate ester were spiked onto the silica gel column and were eluted as described above for the organochlorine pesticides and PCBs. The di-*n*-butyl- and di-*n*-octylphthalates were found in Fraction III (recoveries were 60 and 84 percent, respectively), and the diethylphthalate

TABLE 15. DISTRIBUTION AND PERCENT RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs SPIKED INTO THE LIQUID WASTE NO. 2 EXTRACT (SPIKE LEVEL IS 500 NG FOR PESTICIDES AND 5,000 NG FOR PCBs)

	Percent recovery				
Compounds	Fraction I	Fraction II	Fraction III	Fraction IV	Total
alpha-BHC	72	21	6.5		100
beta-BHC	32	7.2	46		85
gamma-BHC	47	20	41		108
delta-BHC	20	3.9	65		89
Heptachlor	96				96
Aldrin	85				85
Heptachlor epoxide		7.4	78		85
gamma-Chlordane	87	2.5			90
Endosulfan I		4.9	81		86
4,4'-DDE	103				103
Dieldrin			106		106
Endrin			101		101
Endosulfan II			87		87
4,4'-DDD	81	18	9.4		108
Endrin aldehyde			70		70
Kepone				NS	NS
Endosulfan sulfate			80		80
4,4'-DDT	86				86
4,4'-Methoxychlor			78		78
PCB-1016 PCB-1260	99				99

NS -- Not spiked.

was recovered in Fraction IV. The remainder of the di-n-butylphthalate (~50 percent) was also recovered in Fraction IV. Additional experiments will be necessary to assess the impact of the possible interferences from phthalate esters.

6.2.2 Alumina Cleanup

Alumina cleanup is currently recommended by EPA in the Contract Laboratory Program Protocol (12). Hexane/acetone extracts are subjected to neutral alumina cleanup on Super I Woelm or equivalent (Universal Scientific, Atlanta, GA,

TABLE 16. DISTRIBUTION AND PERCENT RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs SPIKED INTO THE LIQUID WASTE NO. 2 EXTRACT (SPIKE LEVEL IS 5,000 NG FOR PESTICIDES AND 50,000 NG FOR PCBs)

Compounds	Percent recovery				
	Fraction I	Fraction II	Fraction III	Fraction IV	Total
alpha-BHC	77	24	3.2		104
beta-BHC	50	7.0	46		103
gamma-BHC	46	26	38		110
delta-BHC	36	6.2	63		105
Heptachlor	76				76
Aldrin	85				85
Heptachlor epoxide		8.5	73		82
gamma-Chlordane	88	3.4			91
Endosulfan I		3.7	88		92
4,4'-DDE	117				117
Dieldrin			131		131
Endrin			86		86
Endosulfan II			98		98
4,4'-DDD	79	18	5.6		103
Endrin aldehyde			89		89
Kepone				NS	NS
Endosulfan sulfate			98		98
4,4'-DDT	92				92
4,4'-Methoxychlor			86		86
PCB-1016 PCB-1260	81				81

NS -- Not spiked.

or equivalent) of activity III (3 g). The organochlorine pesticides and PCBs are eluted with 9 mL hexane. Several reports published in the literature (13, 14) that described alumina cleanup indicated that alumina is more efficient than silica gel and Florisil in removing naturally occurring organic acids, pigments, and other interferences.

TABLE 17. DISTRIBUTION AND PERCENT RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs SPIKED INTO THE SANDY LOAM EXTRACT (SPIKE LEVEL IS 500 NG FOR PESTICIDES AND 5,000 NG FOR PCBs)

	Percent recovery				
Compounds	Fraction I	Fraction II	Fraction III	Fraction IV	Total
alpha-BHC		74	14		88
beta-BHC			98		98
gamma-BHC			85		85
delta-BHC			97		97
Heptachlor	114				114
Aldrin	100				100
Heptachlor epoxide			107		107
gamma-Chlordane	96	8.5			105
Endosulfan I			81		81
4,4'-DDE	125				125
Dieldrin			138		138
Endrin			86		86
Endosulfan II			105		105
4,4'-DDD		63	43		106
Endrin aldehyde			93		93
Kepone				NS	NS
Endosulfan sulfate			106		106
4,4'-DDT	118				118
4,4'-Methoxychlor			97		97
PCB-1016 PCB-1260	94				94

NS -- Not spiked.

TABLE 18. DISTRIBUTION AND PERCENT RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs SPIKED INTO THE NBS SEDIMENT EXTRACT (SPIKE LEVEL IS 500 NG FOR PESTICIDES AND 5,000 NG FOR PCBs)

Compounds	Percent recovery				
	Fraction I	Fraction II	Fraction III	Fraction IV	Total
alpha-BHC	19	69	26		114
beta-BHC			108		108
gamma-BHC	9.4		80		89
delta-BHC			97		97
Heptachlor	90				90
Aldrin	86				86
Heptachlor epoxide			97		97
gamma-Chlordane	80	16			96
Endosulfan I			94		94
4,4'-DDE	107				107
Dieldrin			120		120
Endrin			90		90
Endosulfan II			95		95
4,4'-DDD	11	53	43		107
Endrin aldehyde			86		86
Kepone				NS	NS
Endosulfan sulfate			92		92
4,4'-DDT	79				79
4,4'-Methoxychlor			108		108
PCB-1016 PCB-1260	68				68

NS -- Not spiked.

TABLE 19. DISTRIBUTION AND PERCENT RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs SPIKED INTO THE SOLID WASTE EXTRACT (SPIKE LEVEL IS 500 NG FOR PESTICIDES AND 5,000 NG FOR PCBs)

	Percent recovery				
Compounds	Fraction I	Fraction II	Fraction III	Fraction IV	Total
alpha-BHC	51	48	15		114
beta-BHC			90		90
gamma-BHC		26	58		84
delta-BHC			95		95
Heptachlor	107				107
Aldrin	96				96
Heptachlor epoxide			100		100
gamma-Chlordane	89	11			100
Endosulfan I			96		96
4,4'-DDE	108				108
Dieldrin			109		109
Endrin			121		121
Endosulfan II			92		92
4,4'-DDD	32	52			84
Endrin aldehyde			82		82
Kepone				NS	NS
Endosulfan sulfate			97		97
4,4'-DDT	98				98
4,4'-Methoxychlor			92		92
PCB-1016 PCB-1260	75				75

NS -- Not spiked.

The experiments presented in this section address the applicability of alumina cleanup to the two liquid matrices investigated in this study. In the case of liquid waste No. 1, a 1-mL aliquot of sample extract was loaded to a 3-g neutral Super I Woelm alumina activity III column and was eluted with 10 mL hexane. Activity III was prepared by deactivating alumina with 7 percent water. Recovery of the organochlorine pesticides using alumina cleanup was determined by using standards as well as liquid waste No. 1 extract spiked with 0.5 to 5 pg of the compounds listed in Tables 20 and 21.

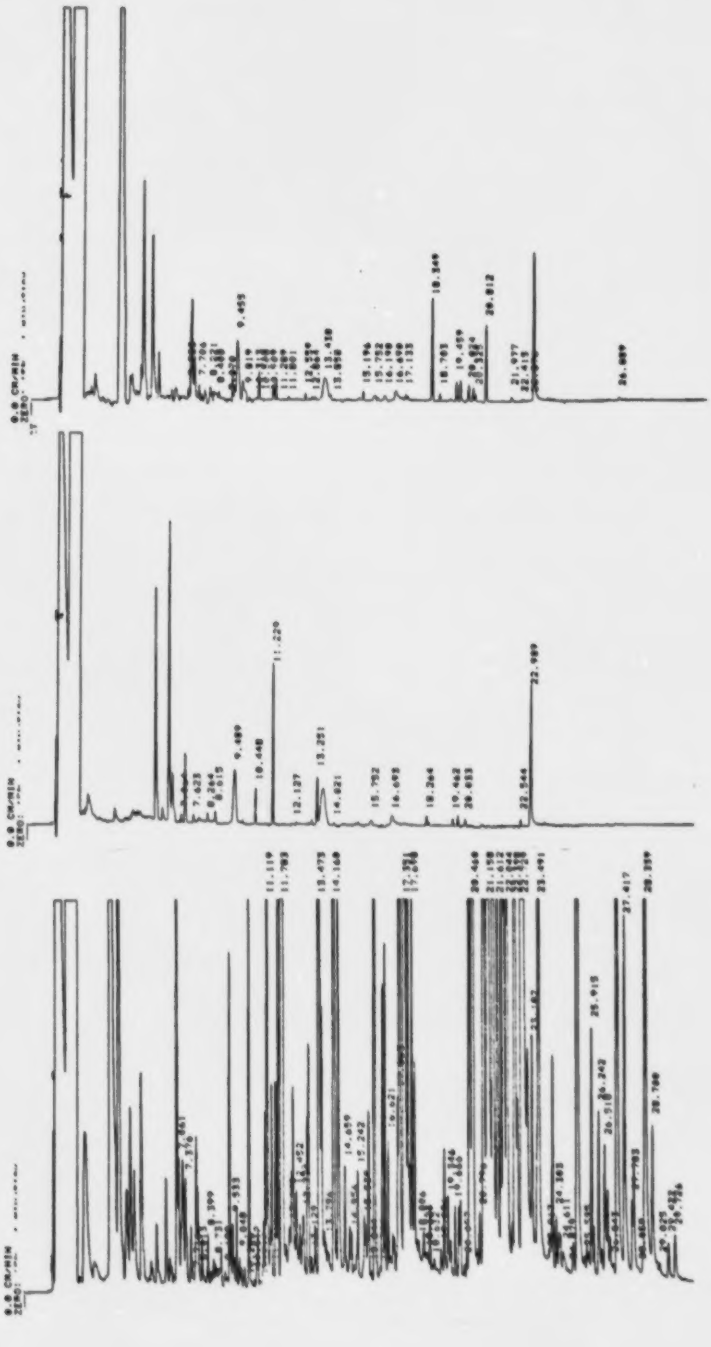

Figure 5. GC/EC chromatograms of the silica gel Fractions I (top), II (middle), and III (bottom) of liquid waste No. 1 extract, analyzed on the 30 m x 0.25 mm ID DB-5 fused-silica capillary column. Temperature program: 100°C (hold at 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; helium at 16 psi.

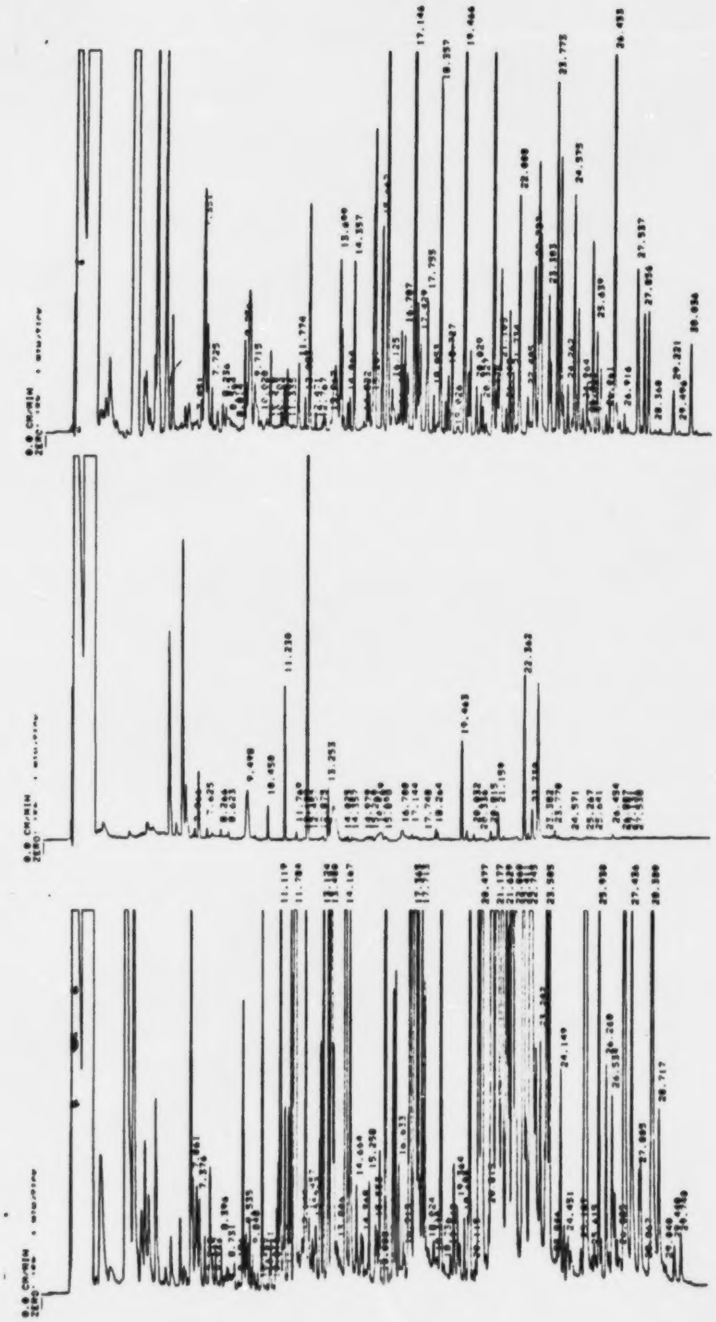

Figure 6. GC/EC chromatograms of the silica gel Fractions I (top), II (middle), and III (bottom) of liquid waste No. 1 extract, spiked with the organochlorine pesticides and PCBs, and analyzed on the 30 m x 0.25 mm ID DB-5 fused-silica capillary column. Temperature program: 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; helium at 16 psi.

TABLE 20. RECOVERY OF SELECTED ORGANOCHLORINE PESTICIDES AFTER CHROMATOGRAPHY ON NEUTRAL SUPER I WOELM ALUMINA[a]

	Amount spiked (μg)	Percent recovery
gamma-BHC	0.5	107
Heptachlor	0.5	101
Aldrin	0.5	98
Dieldrin	0.5	100
Endrin	0.5	107
4,4'-DDT	0.5	107

[a] Activity III (deactivated with 7 percent water).

TABLE 21. RECOVERY OF ORGANOCHLORINE PESTICIDES SPIKED INTO LIQUID WASTE NO. 1 EXTRACT AND SUBJECTED TO ALUMINA CLEANUP

		Hexane elution volume (mL)		
Compound	Spike level (μg)	10	20	50
alpha-BHC	0.5	79	86	102
beta-BHC	0.5	a	a	a
gamma-BHC	0.5	34	104	a
delta-BHC	0.5	23	0	15
Heptachlor	0.5	79	69	99
Aldrin	0.5	99	82	a
Heptachlor epoxide	0.5	4.6	80	112
Endosulfan I	0.5	18	74	102
Dieldrin + 4,4'-DDE	1.0	69	58	126
Endrin	1.0	0.6	72	111
Endosulfan II	1.0	2.3	3.6	0
4,4'-DDD	1.0	26	5.7	110
Endrin aldehyde	1.0	0.7	5.7	18
Kepone	1.0	7.6	0	0
Endosulfan sulfate	1.0	2.5	0	a
4,4'-DDT	1.0	76	62	90
4,4'-Methoxychlor	5.0	0.4	0	142

[a] Recovery could not be determined because of high background levels of electron-capturing compounds.

The recovery data in Table 20 were obtained with standards and indicate excellent recoveries for the 6 compounds tested. When alumina cleanup was attempted on liquid waste No. 1 extract, 12 of the 17 spiking compounds gave unacceptable recoveries. The experiment was therefore repeated but the volume of hexane was increased from 10 mL to 20 mL and then to 50 mL. The results of these experiments are presented in Table 21. Although increasing the hexane elution volume improved recoveries for many of the organochlorine pesticides, the amount of interfering compounds eluted from the alumina column was also greatly increased (Figures 7 through 9).

When liquid waste No. 2 was subjected to alumina cleanup, recoveries of 16 organochlorine pesticides were found to be >79 percent (Table 22). Kepone was not recovered at all.

Experimental results reported in Table 21 show that an increase in the hexane elution volume from 10 mL to 50 mL resulted in improved recoveries for alpha-BHC, heptachlor, heptachlor epoxide, endosulfan I, dieldrin, 4,4'-DDE, endrin, 4,4'-DDD, and 4,4'-methoxychlor. However, the amount of interfering compounds eluted from the alumina column was also greatly increased, and this precluded the quantification of beta- and gamma-BHC, aldrin, and endosulfan sulfate. An alternative approach to improving recoveries of the test compounds would be to change the degree of deactivation of the alumina while keeping the elution volume of hexane constant.

Alumina (Super I Woelm, neutral alumina, 3 g) deactivated with 7, 10, 15, and 19 percent water was tested with a pesticide standard. Recoveries are presented in Table 23. The results indicate that in order to obtain quantitative recoveries for all of the 18 organochlorine pesticides, alumina deactivated with 19 percent water is required. Only Kepone (27 percent recovery) and endrin aldehyde (50 percent recovery) gave still low recoveries. Liquid waste No. 1 was also tested by using alumina deactivated with 15 percent and 19 percent water. The pertaining GC/EC chromatograms are shown in Figures 10 and 11. Although the alumina deactivated with 19 percent water gives higher recoveries for the organochlorine pesticides of interest, we were not able to quantify many of the spiked compounds because of material coextracted from the matrix. When a similar experiment was performed with liquid waste No. 2, the same conclusion was reached. However, in that case, the extract was applied to the alumina column in benzene, and the compound elution patterns were somewhat different.

No experiments were performed to determine recoveries of PCBs by using the alumina cleanup procedure described above.

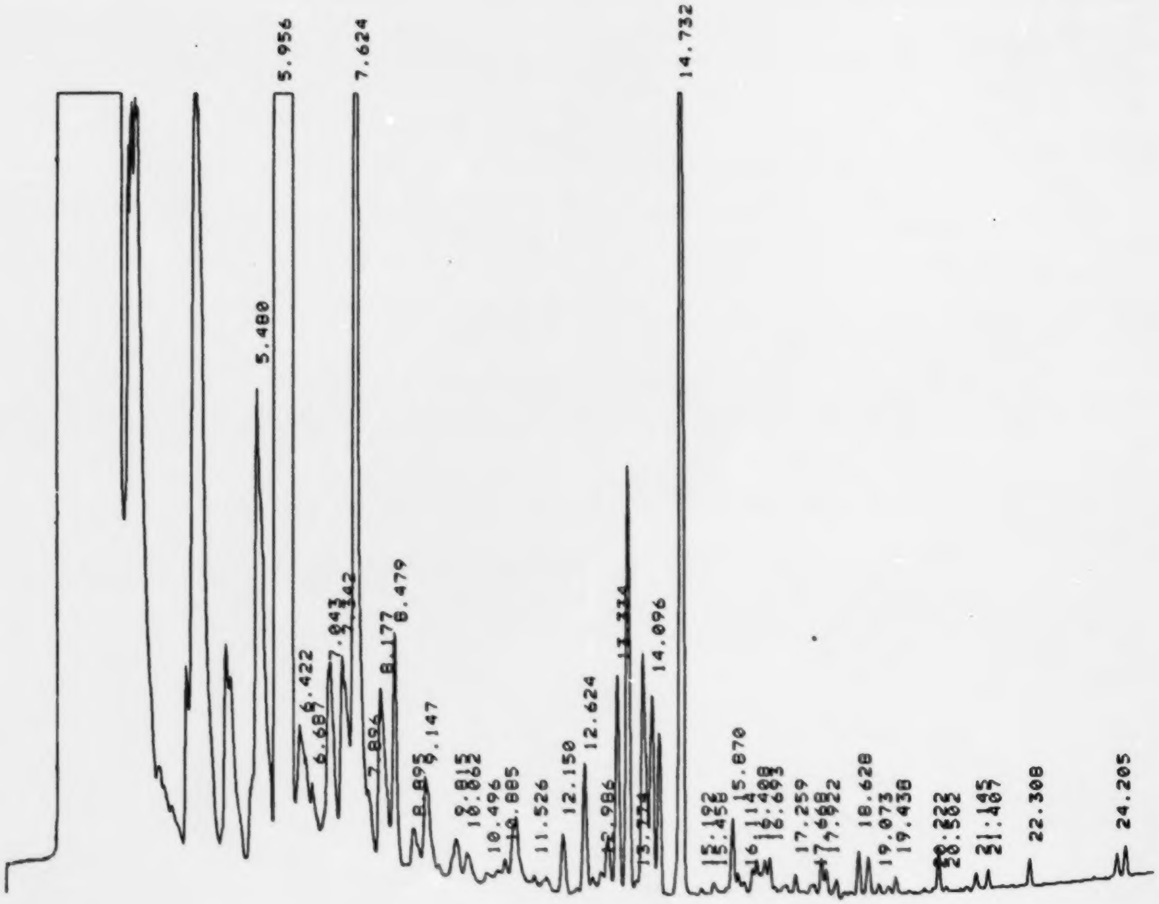

Figure 7. GC/EC chromatogram of the liquid waste No. 1 extract after alumina cleanup (10 mL hexane).

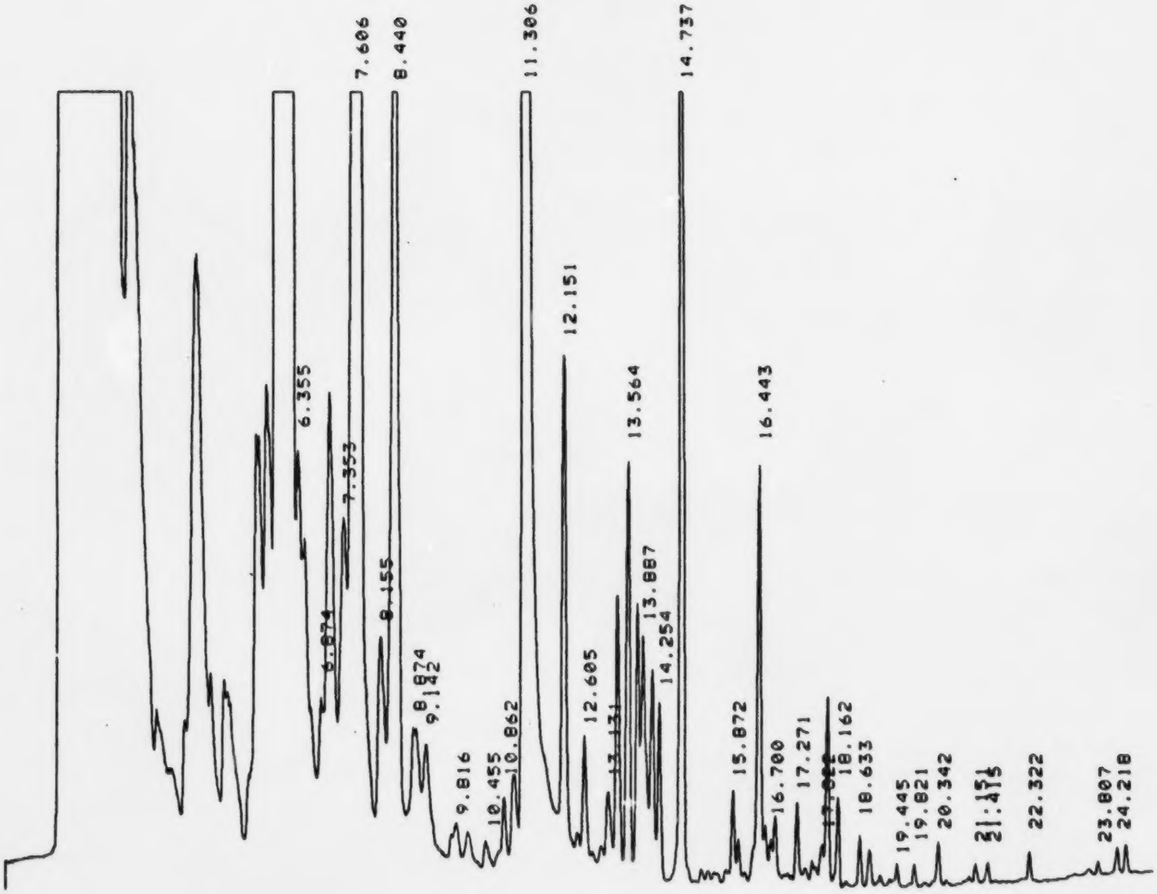

Figure 8. GC/EC chromatogram of the liquid waste No. 1 extract after alumina cleanup (20 mL hexane).

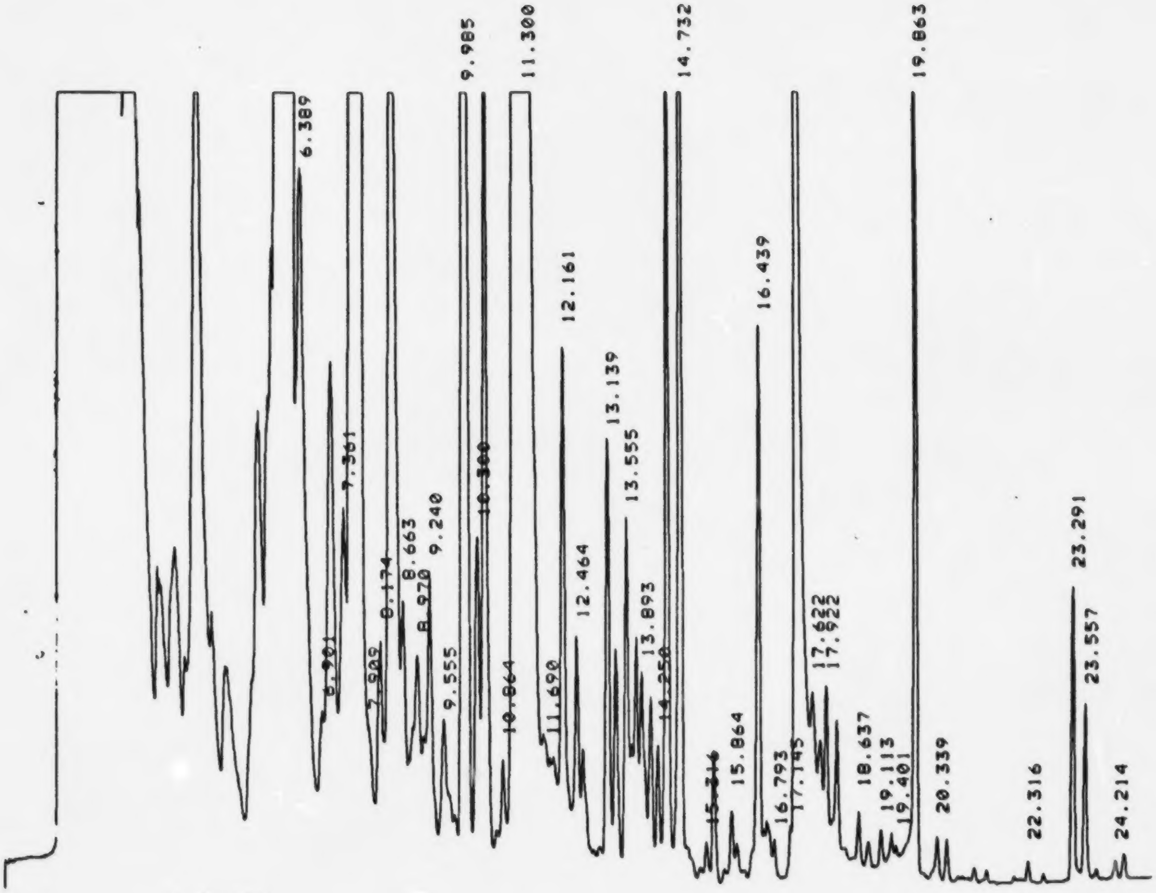

Figure 9. GC/EC chromatogram of the liquid waste No. 1 extract after alumina cleanup (50 mL hexane).

TABLE 22. RECOVERY OF THE ORGANOCHLORINE PESTICIDES SPIKED INTO LIQUID WASTE NO. 2 AND SUBJECTED TO ALUMINA CLEANUP

Compound	Spike level (μg)	Percent recovery
alpha-BHC	5.0	100
beta-BHC	5.0	103
gamma-BHC	5.0	87
delta-BHC	5.0	94
Heptachlor	5.0	82
Aldrin	5.0	89
Heptachlor epoxide	5.0	90
Endosulfan I	5.0	99
Dieldrin + 4,4'-DDE	10.0	93
Endrin	10.0	88
Endosulfan II	10.0	80
4,4'-DDD	10.0	94
Endrin aldehyde	10.0	79
Kepone	10.0	3
Endosulfan sulfate	10.0	90
4,4'-DDT	10.0	88
4,4'-Methoxychlor	50.0	94

From the data presented in this section it can be concluded that alumina cleanup may be useful, although this depends on the type of matrix and on interfering coextracted materials. The usefulness of the alumina cleanup results primarily from the fact that by controlling the activity of the alumina adsorbent, one could recover all the organochlorine pesticides in one fraction while polar compounds such as phthalate esters that may interfere with the gas chromatographic analysis would be left behind. However, to the best knowledge of the authors, there are no reports that indicate that the organochlorine pesticides and PCBs can be fractionated and recovered quantitatively by using alumina chromatography.

6.2.3 Silica Gel/Celite

The silica gel/Celite cleanup procedure of Armour and Burke (15) was tested by using a mixture of 5 g acid-washed Celite and 20 g of silica gel deactivated with 3 percent water. A mixed pesticide standard (0.5 to 1.0 μg) and 5 μg of PCB-1016 and PCB-1260 were added to the top of the column in 4 mL hexane. Fractions 1, 2, and 3 were eluted from the column with 250 mL hexane, 200 mL acetonitrile:hexane:methylene chloride (1:19:80), and 200 mL methylene chloride, respectively. The fractions were concentrated in a Kuderna-Danish apparatus, and the solvent was exchanged to hexane and brought up to 10 mL. Recoveries are given in Table 24.

TABLE 23. ALUMINA CLEANUP RECOVERIES

Compound	Amount spiked (μg)	Percent deactivation			
		7	10	15	19
alpha-BHC	0.5	86.0	92.9	107	99.8
beta-BHC	0.5	0	0	101	100
gamma-BHC	0.5	70.6	88.0	105	101
delta-BHC	0.5	0	0	7.8	99.0
Heptachlor	0.5	77.1	94.4	109	98.3
Aldrin	0.5	84.5	99.7	106	102
Heptachlor epoxide	0.5	8.8	59.2	107	101
Endosulfan I	0.5	37.7	84.9	102	99.5
Dieldrin + 4,4'-DDE	1.0	74.3	70.3	103	99.0
Kepone	1.0	10.5	17.5	8.7	26.7
Endrin	1.0	5.2	49.8	106	99.8
Endosulfan II	1.0	0	1.4	0	96.1
4,4'-DDD	1.0	64.4	96.4	105	102
Endrin aldehyde	1.0	1.1	22.2	30.4	50.2
Endosulfan sulfate	1.0	0	2.8	0	92.3
4,4'-DDT	1.0	57.9	86.6	89.6	96.0
4,4'-Methoxychlor	5.0	0	0	77.7	94.0

1 mL pesticide standard in hexane added to 3-g alumina column; compounds eluted with 10 mL hexane.

This fractionation procedure was evaluated because it was reported to allow separation of the PCBs from the organochlorine pesticides listed in Method 8080. Specifically, the PCBs are eluted from the silica gel/Celite column with petroleum ether prior to the elution of the pesticides with acetonitrile, hexane, and methylene chloride. The data presented in Table 24 do indicate some degree of separation between the PCBs and the organochlorine pesticides; however, the recoveries are low for gamma-BHC, 4,4'-DDE, endrin aldehyde, and the PCBs, and beta-BHC, Kepone, and 4,4'-DDD could not be recovered at all.

6.2.4 Florisil/Charcoal

Florisil and charcoal column chromatography were combined by using the method of Berg et al. (16). Twenty-one grams of activated Florisil were packed into an 11-mm ID column and were topped with 2 to 3 cm sodium sulfate (8g). A mixed pesticide standard (0.5 to 1.0 pg each) and 5 μg of PCB-1016 and 5 μg PCB-1260 in 4 mL hexane were added to the top of the column. Two hundred mL hexane were passed through the column, were concentrated, exchanged with acetone, and set aside for the charcoal cleanup. Next, Fractions 1, 2, and 3 were eluted from the Florisil column with 200 mL each of 6 percent ethyl ether/hexane, 15 percent ethyl ether/hexane, and 50 percent ethyl ether/hexane, respectively.

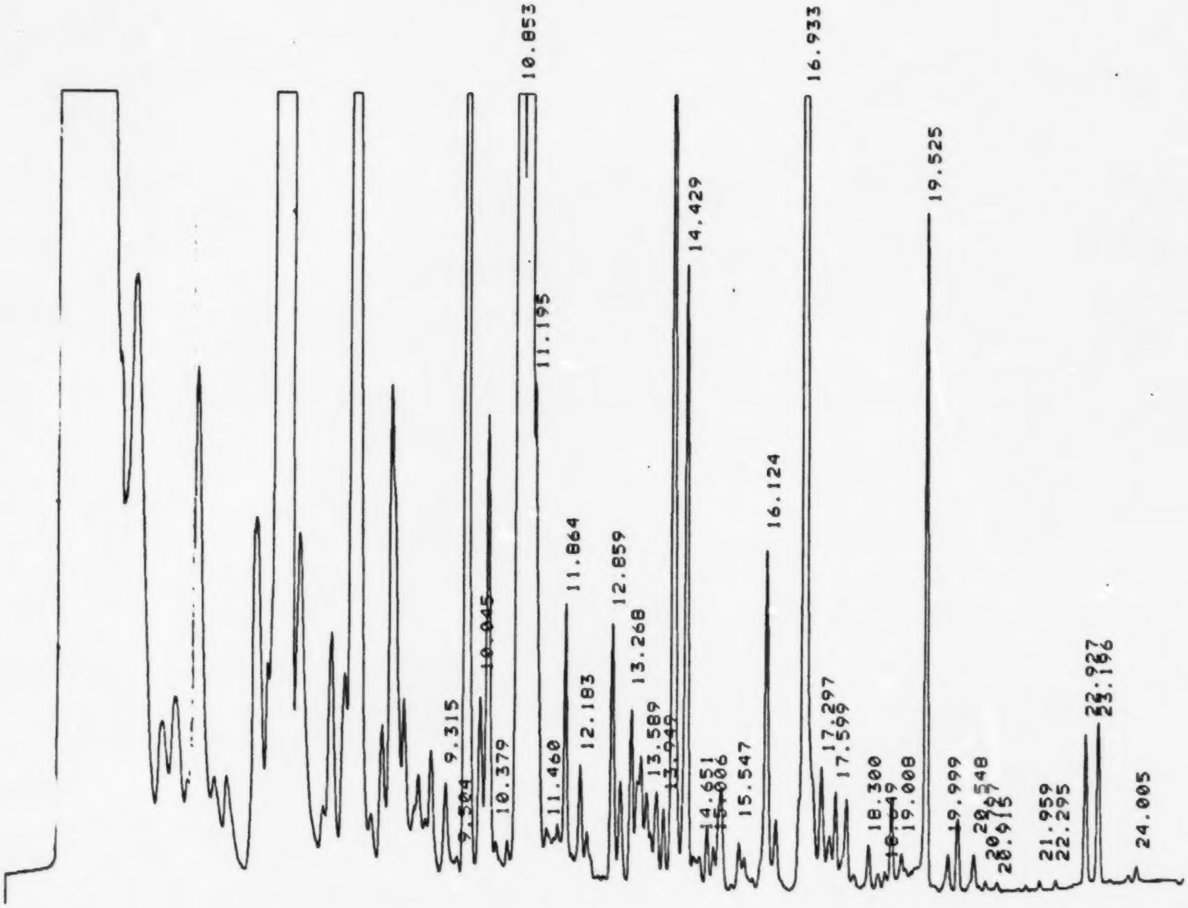

Figure 10. GC/EC chromatogram of the liquid waste No. 1 extract after cleanup on alumina deactivated with 15 percent water.

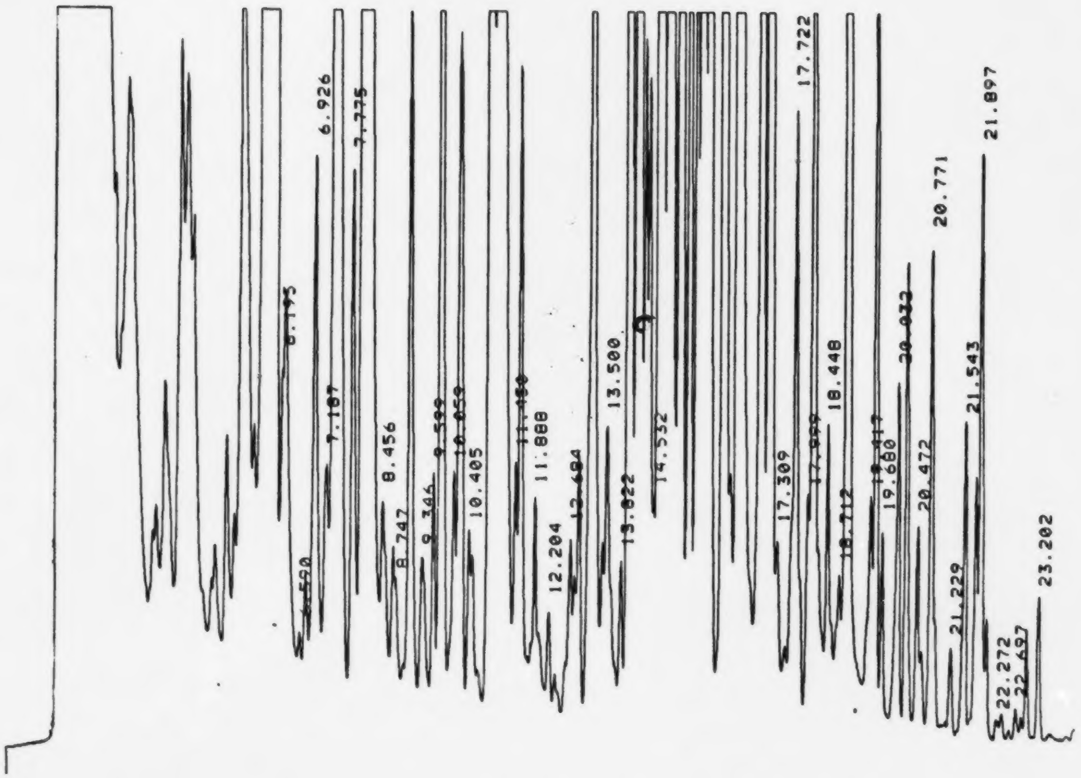

Figure 11. GC/EC chromatogram of liquid waste No. 1 extract after cleanup on alumina deactivated with 19 percent water.

TABLE 24. ELUTION PATTERNS AND PERCENT RECOVERIES FROM THE SILICA GEL/CELITE COLUMN CLEANUP

		Fraction[a]			
Compound	Amount added (μg)	1	2	3	Total
alpha-BHC	0.5		53.9		53.9
beta-BHC	0.5				0
gamma-BHC	0.5		37.3		37.3
delta-BHC	0.5				b
Heptachlor	0.5	26.2	54.0		80.2
Aldrin	0.5	64.4			64.4
Heptachlor epoxide	0.5		63.6	2.5	66.1
Endosulfan I	0.5		58.1		58.1
Dieldrin	1.0		NI	3.0	--
4,4'-DDE	1.0		19.8		19.8
Endrin	1.0		65.8	0.9	66.7
Endosulfan II	1.0			7.1	7.1
4,4'-DDD	1.0				0
Endrin aldehyde	1.0		45.5		45.5
Kepone	1.0				0
Endosulfan sulfate	1.0		67.8		67.8
4,4'-DDT	1.0		83.9		83.9
4,4'-Methoxychlor	5.0		58.3		58.3
PCB-1016	5.0	26.9	12.7	1.9	41.5
PCB-1260	5.0	42.1	0.7		42.8

NI -- Peak not integrated.

[a] Fraction 1 eluted with 250 mL hexane; Fraction 2 eluted with 200 mL acetonitrile:hexane:methylene chloride (1:19:80); Fraction 3 eluted with 200 mL methylene chloride.

[b] Not able to quantify because of interference.

Activated charcoal (1.1 g) was packed into a 6-mm ID column and was washed with acetone. The first eluate from the Florisil cleanup was added to the top of the charcoal column and was eluted with 90 mL 25-percent acetone in benzene (Fraction 4) and then with 60 mL benzene (Fraction 5). Elution patterns and percent recoveries are given in Table 25.

TABLE 25. ELUTION PATTERNS AND PERCENT RECOVERIES FROM THE FLORISIL/CHARCOAL CLEANUP

Compound	Amount added (μg)	Fraction[a] 1	2	3	4	5	Total
alpha-BHC	0.5	46.9	3.8				50.7
beta-BHC	0.5	68.7					68.7
gamma-BHC	0.5	82.2	3.0				85.2
delta-BHC	0.5	43.6	18.0				61.6
Heptachlor	0.5	30.6			41.6		72.2
Aldrin	0.5	29.7			50.1		79.8
Heptachlor epoxide	0.5	73.2					73.2
Endosulfan I	0.5	38.3	42.3				80.6
Dieldrin[b]	1.0	23.6	53.6		21.0		98.2
4,4'-DDE[b]	1.0						
Endrin	1.0	3.1	58.4				61.5
Endosulfan II	1.0			97.0			97.0
4,4'-DDD	1.0	61.7	3.9				65.6
Endrin aldehyde	1.0	1.1	15.0	18.3			34.4
Kepone	1.0			8.9			8.9
Endosulfan sulfate	1.0			88.0			88.0
4,4'-DDT	1.0	37.1	22.9				60.0
4,4'-Methoxychlor	5.0	35.3					35.3
PCB-1016	5.0	40.9				60.2	100
PCB-1260	5.0	47.9			27.9	90.9	167

[a] Fractions 1, 2, and 3 eluted from the Florisil column with 200 mL each of 6, 15, and 50 percent ethyl ether in hexane, respectively. Fractions 4 and 5 were eluted from the charcoal column with 90 mL 25-percent acetone in benzene and with 60 mL benzene, respectively. They both represent the first eluate from the Florisil column (see text).

[b] Dieldrin and 4,4'-DDE are coeluting.

The fractionation scheme with Florisil/charcoal resulted in elution of the organochlorine pesticides and PCBs in five fractions. The first compounds to elute from the Florisil column are heptachlor, aldrin, dieldrin, and the PCBs, and they are further fractionated on the charcoal column. The remainder of the organochlorine pesticides elutes in Fractions 1, 2, and 3. The more polar compounds such as endosulfan II and endosulfan sulfate are recovered quantitatively whereas endrin aldehyde and Kepone are only partially recovered. No further attempts were made to evaluate this fractionation scheme since the Florisil/charcoal did not seem to be superior to the (simpler) silica gel cleanup.

6.2.5 Sulfur Cleanup

Elemental sulfur, if present, will give large peaks near the solvent area which often mask the region from the solvent peak to aldrin when analysis of the organochlorine pesticides is performed on the 1.8-m glass column packed with 1.5 percent OV-17/1.95 percent OV-210 on Chromosorb WHP. The methods available for the removal of elemental sulfur have been reviewed in Appendix A. The procedure selected for evaluation in this study consists of the non-destructive treatment of the extract with tetrabutylammonium sulfite (17). This lipophilic ion pair rapidly converts sulfur to thiosulfate.

Sulfur is soluble to some extent in hexane, but not in water, and sodium sulfite is soluble in water, but not in hexane; therefore, the reaction between these compounds that results in thiosulfate formation occurs very slowly. When the tetrabutylammonium ion is present, a lipophilic ion pair is formed with the sulfite, and added 2-propanol favors the solvation of this ion pair in the nonpolar phase which allows a momentary reaction with the sulfur. When excess sodium sulfite is present the sulfur removal is quantitative.

To investigate the removal of sulfur from sample extracts, five matrix extracts, identified below, were split as follows: 2-mL aliquots of each matrix extract (liquid waste No. 1, liquid waste No. 2, sandy loam, NBS sediment, and solid waste) were subjected to a sulfur cleanup with the tetrabutylammonium (TBA)-sulfite reagent; other 2-mL aliquots from the five matrix extracts were first spiked with 100 ng or 1000 ng of the organochlorine pesticides and then were subjected to a sulfur cleanup by the same procedure; finally, other 2-mL aliquots of the five matrix extracts were spiked with the organochlorine pesticides and sulfur (300 ug) and were then subjected to the sulfur cleanup procedure.

The TBA-sulfite reagent was prepared from 3.39 g tetrabutyl ammonium hydrogen sulfate and 100 mL water. To remove any organic impurities, this solution was extracted 3 times with 20 mL hexane. One-milliliter aliquots of the TBA sulfate solution were then transferred to individual vials, and 250 mg of crystalline sodium sulfite were added to each vial. It is very important that excess sulfite is always present; otherwise, the removal of sulfur is not complete, and the reaction with the TBA-sulfite reagent has to be repeated.

The matrix extracts (in hexane) were shaken with 2-propanol (1 mL) and the TBA-sulfite reagent (1 mL) for at least 1 min. Water (5 mL) was added, and the vial containing the extract and reagent was shaken for another min on a Vortex.

The rationale for performing this experiment was two-fold: to determine if the removal of sulfur is affected by matrix interferences and to determine if the organochlorine pesticides are recovered quantitatively. In addition to the 5 matrix extracts, 3 pesticides standards (one of them containing 300 pg sulfur) were also reacted with the TBA-sulfite reagent to determine how well the pesticides are recovered in the absence of matrix interferences.

The results are presented in Tables 26 and 27. It is clear from the data in Table 26 that all the organochlorine pesticides are recovered quantitatively. Because of matrix interferences, quantification of many organochlorine pesticides was not possible (Table 27). However, from examining the GC/EC chromatograms (Figures 12 and 13) it appears that sulfur was removed quantitatively, regardless of the matrix; thus, the procedure is recommended for incorporation into the Method 8080 protocol. The observations which follow were made during this study and are considered worthy of note:

- All extracts should be examined visually for the presence of sulfur; when sulfur crystals are present (yellow color), the extract should be removed with a syringe or disposable pipet, transferred to another vial, and subjected to the TBA-sulfite procedure.
- Even if sulfur crystals are not visible in the extract but sulfur appears to be present according to the GC/EC analysis (see chromatograms in Figures 12 and 13), then the extract should be reacted with the TBA-sulfite reagent and reanalyzed.

6.3 GAS CHROMATOGRAPHY

The analysis of the organochlorine pesticides and PCBs listed in Method 8080 by gas chromatography with electron capture detection has been reviewed in Appendix A, Section 2.4. Basically, the compounds are separated on a packed or a capillary column, either under isothermal or temperature-programmed conditions, and are identified by matching the retention times of the peaks in the sample with those derived from the calibration standard, which was analyzed under the same conditions as the sample. A second or even a third column is required to confirm compounds by gas chromatography to minimize or to avoid misinterpretation of compound identity.

The packed-column chromatographic determination of the organochlorine pesticides, although widely used, does not allow complete separation of the pairs beta- and gamma-BHC, 4,4'-DDE and dieldrin, and 4,4'-DDD and endosulfan II. Furthermore, multicomponent mixtures such as toxaphene and PCBs are resolved into much fewer components with a packed column than with a capillary column.

This section summarizes the results of a series of experiments performed with the intent of identifying a set of conditions that will allow adequate separation of the 19 organochlorine pesticides from the PCB components. Four columns, two packed (1.5 percent OV-17/1.95 percent OV-210 and 3 percent OV-1) and two capillary (DB-5 and SPB-608), were evaluated. Standards containing all 19 organochlorine pesticides were analyzed separately from the PCBs and toxaphene mixtures. Mixtures containing the organochlorine pesticides at 0.05 to 0.50 ng/uL and PCBs or toxaphene at 0.05, 0.10, 0.50, and 1.0 ng/µL were also analyzed to determine a threshold value at which interferences from the multicomponent mixture can be ignored.

TABLE 26. RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs SUBJECTED TO THE TBA-SULFITE PROCEDURE (STANDARDS ONLY, IN HEXANE)

	Percent recovery	
Compound	Without sulfur added[a]	With sulfur added[b]
alpha-BHC	110	103
beta-BHC	106	99
gamma-BHC	112	108
delta-BHC	100	86
Heptachlor	103	97
Aldrin	104	99
Heptachlor epoxide	107	101
gamma-Chlordane	98	100
Endosulfan I	108	106
4,4'-DDE and Dieldrin	91	103
Endrin	87	89
Endosulfan II	99	99
4,4'-DDD	103	106
Endrin aldehyde	10	8
Kepone	107	63
Endosulfan sulfate	94	93
4,4'-DDT	86	90
4,4'-Methoxychlor	85	68
Toxaphene	93	83
PCB-1016	108	88
PCB-1260	87	68

[a] Value given is the average of two determinations.
[b] Single determination.

A summary of the organochlorine pesticides retention times is given in Table 28. Chromatograms of individual as well as composite mixtures of organochlorine pesticides and PCBs are given in Figures 14 to 18. Additional chromatograms from the capillary column analysis are given in Appendix B.

It can be seen that the separation of the organochlorine pesticides and PCBs cannot be achieved by using the packed column (1.5 percent OV-17/195-percent OV-210) recommended by the EPA in Method 8080. Likewise, toxaphene causes a shift in the baseline when it is present at concentrations twice as high as some of the organochlorine pesticides.

TABLE 27. RECOVERIES OF THE ORGANOCHLORINE PESTICIDES SPIKED INTO VARIOUS MATRICES AND SUBJECTED TO THE TBA-SULFITE PROCEDURE

Compound	Liquid Waste 1		Liquid Waste 2		Sandy loam		NBS sediment		Solid Waste 1	
	Without sulfur	With sulfur	Without sulfur	With sulfur	Without sulfur	With sulfur	Without sulfur	With sulfur	Without sulfur	With sulfur
alpha-BHC	61	56	49	13	95	75	87	84	76	66
beta-BHC	23	22	7.5	0	96	97	83	85	85	0
gamma-BHC	a	a	45	0	72	41	90	120	105	27
delta-BHC	a	a	0	0	67	39	71	61	0	0
Heptachlor	70	80	94	104	113	114	99	105	59	57
Aldrin	a	a	109	0	103	106	66	76	a	a
Heptachlor epoxide	a	a	a	a	103	98	91	74	40	29
gamma-Chlordane	83	93	26	0	105	104	121	81	a	a
Endosulfan I	92	79	a	a	105	98	96	82	a	a
4,4'-DDE + Dieldrin	77	67	75	71	81	80	78	73	a	a
Endrin	a	a	115	79	128	132	92	86	a	a
Endosulfan II	a	a	49	4.2	110	103	87	65	11	0
4,4'-DDD	a	a	41	0	102	85	65	60	0	0
Endrin aldehyde	45	68	89	8.9	33	83	0	49	0	0
Endosulfan sulfate	a	a	42	0	39	a	49	a	0	0
4,4'-DDT	67	102	94	94	118	155	a	a	a	a
4,4'-Methoxychlor	b	b	b	b	b	b	b	b	b	b

[a] Unable to quantify due to interfering compound(s).

[b] Unable to quantify 4,4'-methoxychlor because of poor chromatography on the DB-5 column at the time the experiment was performed.

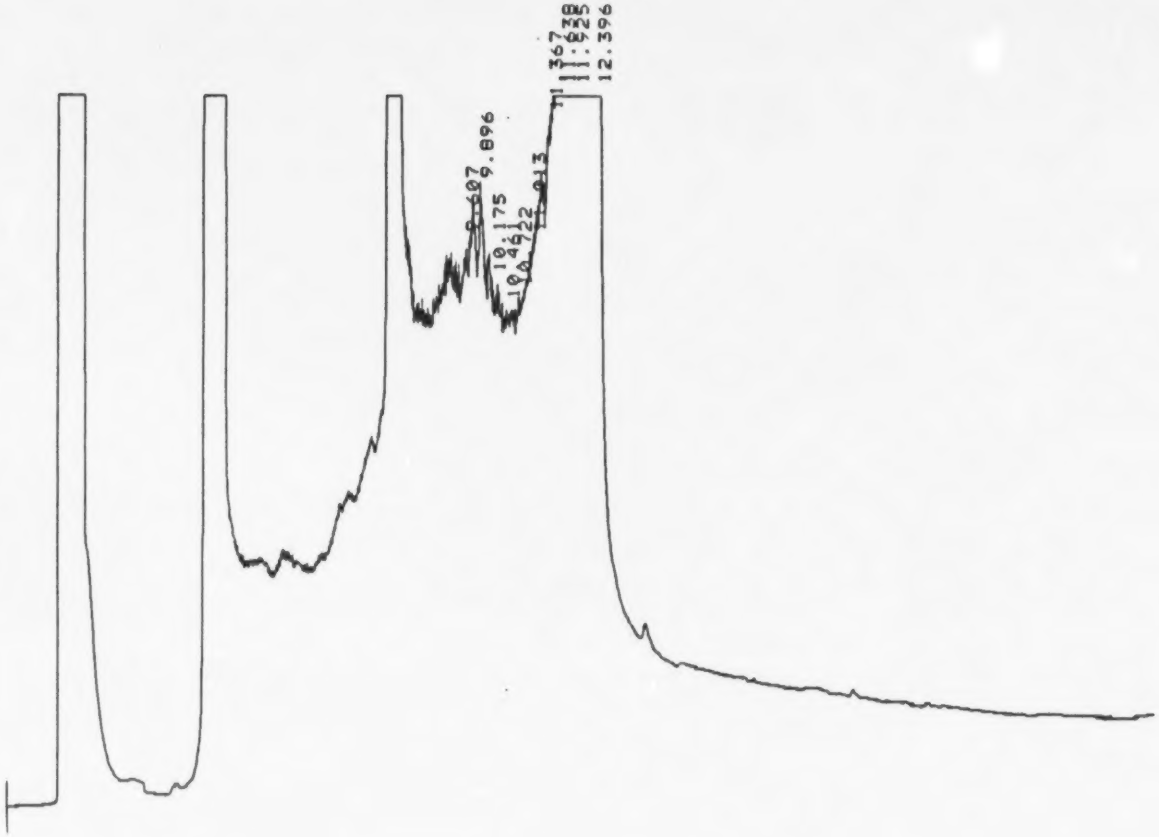

Figure 12. GC/EC chromatogram of the sulfur standard (concentration 300 μg/mL) before reaction with the TBA-sulfite reagent.

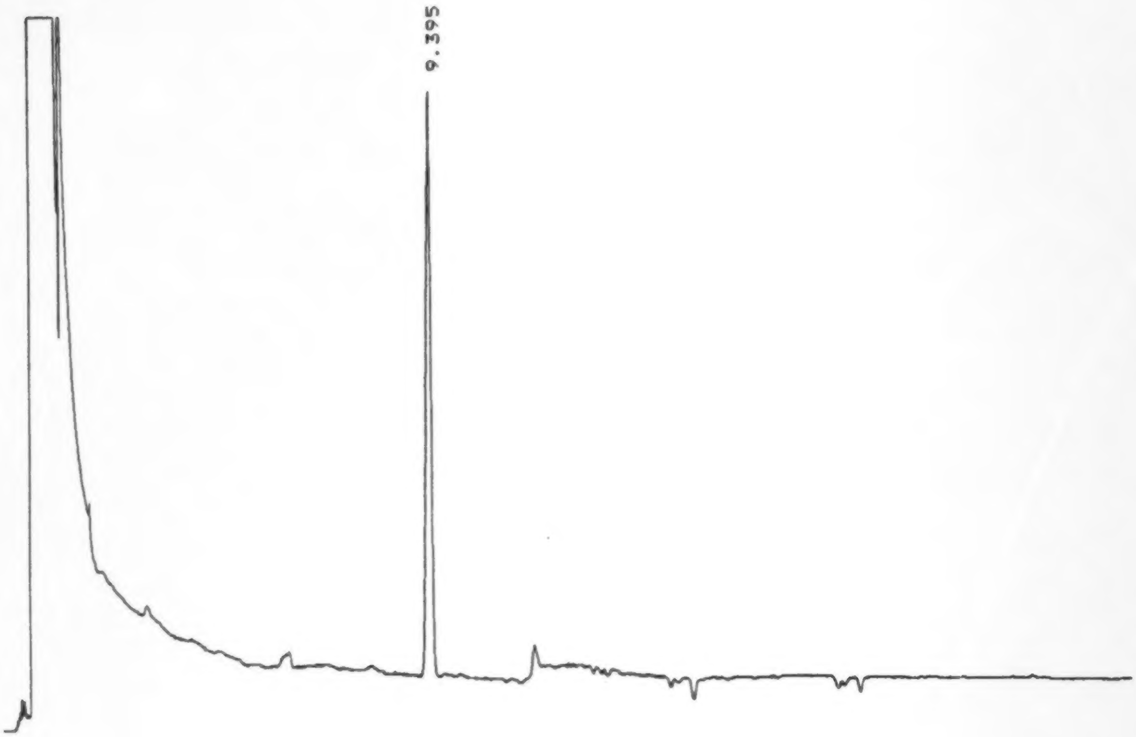

Figure 13. GC/EC chromatogram of the sulfur standard after reaction with the TBA-sulfite reagent.

TABLE 28. SUMMARY OF ORGANOCHLORINE PESTICIDE RETENTION TIMES (MIN)

Compound	Packed columns		Capillary columns		
			DB-5^c		SPB-608^d
	1.5 percent OV-17^a + 1.95 percent OV-210	3 percent OV-1^b	(1)	(2)	
alpha-BHC	1.54	1.54	6.52	12.29	9.46
beta-BHC	2.29	1.66	7.28	13.13	11.33
gamma-BHC	1.97	1.90	7.49	13.37	10.97
delta-BHC	2.69	1.83	8.19	14.14	12.73
Heptachlor	2.41	3.07	9.83	15.91	12.46
Aldrin	2.93	3.35	11.02	17.16	13.76
gamma-Chlordane	5.00	5.53	13.26	19.48	16.70
Heptachlor epoxide	4.49	4.80	12.43	18.60	15.98
Endosulfan I	5.68	6.06	13.72	19.94	17.40
4,4'-DDE	6.65	7.30	14.68	20.83	18.36
Dieldrin	6.99	7.22	14.68	20.91	18.60
Endrin	8.52	8.05	15.36	21.71	19.96
Endosulfan II	10.48	8.45	16.20	22.75	20.69
4,4'-DDD	10.37	9.34	15.80	22.38	20.53
Endrin aldehyde	14.01	9.45	16.51	22.75	21.90
Kepone	8.33	10.66	16.72	23.01	D
Endosulfan sulfate	17.05	11.00	17.39	23.64	22.54
4,4'-DDT	12.51	12.44	17.59	23.79	21.72
4,4'-Methoxychlor	24.71	19.51	19.77	25.94	24.90
alpha-Chlordane	5.47	6.18	13.48	NA	17.31

D = degraded on column.

[a] GC operating conditions are given in Table 29.
[b] GC operating conditions are given in Table 30.
[c] GC operating conditions are given in Table 31.
[d] GC operating conditions are given in Table 32.
NA -- data not available.

TABLE 29. GAS CHROMATOGRAPHIC CONDITIONS FOR THE DETERMINATION OF PESTICIDES AND PCBs BY GC/EC USING A 1.5-PERCENT OV-17/1.95-PERCENT OV-210 COLUMN

Chromatograph:	Varian Vista 6000 Gas Chromatograph with a Varian 402 Data System and electron capture detector (constant current-pulsed frequency)
Column:	1.8 m x 2 mm ID glass column 1.5 percent OV-17 + 1.95 percent OV-210 on Chromsorb W HP 100/120 mesh
Temperatures:	Oven 190°C, isothermal Injector 225°C Detector 350°C
Carrier Gas:	Nitrogen at 30 mL/min
Detector:	Range 10 Attenuation 16
Autosampler:	Varian Series 8000; 2 µL injection volume

TABLE 30. GAS CHROMATOGRAPHIC CONDITIONS FOR THE DETERMINATION OF PESTICIDES AND PCBs BY GC/EC USING A 3-PERCENT OV-1 COLUMN

Chromatograph:	Varian Vista 6000 Gas Chromatograph with a Varian 402 Data System and electron capture detector (constant current-pulsed frequency)
Column:	1.8 m x 2 mm ID glass column 3 percent OV-1 on Supelcoport 100/120 mesh
Temperatures:	Oven 182°C, isothermal Injector 225°C Detector 350°C
Carrier Gas:	Nitrogen at 30 mL/min
Detector:	Range 10 Attenuation 16
Autosampler:	Varian Series 8000; 2 µL injection volume

TABLE 31. GAS CHROMATOGRAPHIC CONDITIONS FOR THE DETERMINATION OF PESTICIDES AND PCBs BY GC/EC USING A DB-5 FUSED-SILICA CAPILLARY COLUMN

Chromatograph:	Varian Vista 6000 Gas Chromatograph with a Varian 402 Data System and electron capture detector (constant current-pulsed frequency)
Column:	Fused-silica capillary column 30 m x 0.25 mm ID DB-5, 0.25 μm coating thickness
Temperatures:	Oven (1) 160°C (hold 2 min) to 270°C (hold 1 min) at 5°C/min (2) 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C Injector 225°C Detector 350°C
Carrier Gas:	(1) Nitrogen at 20 psi (2) Helium at 16 psi
Detector:	Range 10 Attenuation 16
Splitless Injection:	Split flow -- 35 mL/min Sweep flow -- off Splitless time: 30 sec
Autosampler:	Varian Series 8000; 2 μL injection volume

In contrast to the packed column, the DB-5 capillary column gives excellent resolution of the organochlorine pesticides and of the multicomponent mixtures such as toxaphene, PCBs, and the technical chlordane. We have investigated two capillary columns and, based on the retention time data given in Table 28, have concluded that, except for Kepone, both columns can be used for the analysis of the organochlorine pesticides including toxaphene and chlordane, and for the PCBs. Because the PCBs are extracted and isolated along with the organochlorine pesticides, these two types of contaminants need to be separated from each other to avoid cross-interference. We found that the resolving power of the capillary column, although significantly greater than that of the packed column, is not sufficient to distinguish between these two groups of compounds. Therefore, we have evaluated the separation of the organochlorine pesticides and PCBs by column chromatography prior to the gas chromatographic analysis.

TABLE 32. GAS CHROMATOGRAPHIC CONDITIONS FOR THE DETERMINATION OF PESTICIDES AND PCBs BY GC/EC USING AN SPB-608 FUSED-SILICA CAPILLARY COLUMN

Chromatograph:	Varian Vista 6000 Gas Chromatograph with a Varian 402 Data System and electron capture detector (constant current-pulsed frequency)
Column:	Fused-silica capillary column 30 m x 0.25 mm ID SPB-608, 0.25 µm coating thickness
Temperatures:	Oven 160°C (2 min hold), then temperature programmed at 5°C/min to 290°C (1 min hold) Injector 225°C Detector 350°C
Carrier Gas:	Nitrogen at 20 psi
Detector:	Range 10 Attenuation 16
Splitless Injection:	Split flow -- 35 mL/min Sweep flow -- off Splitless time: 30 sec
Autosampler:	Varian Series 8000; 2 µL injection volume

Tables 33 through 36 present a comparison of four gas chromatographic analyses of the same "sample" (in this case, there are four fractions collected from a silica gel column) that was analyzed on four different gas chromatographic columns. The "sample" was obtained by fractionating a standard of known concentration of organochlorine pesticides (concentration 1 was 0.5 µg for the BHC isomers, heptachlor, aldrin, heptachlor epoxide, and endosulfan I, and 1.0 µg for the remainder of the organochlorine pesticides, except for 4,4'-methoxychlor which was 5.0 µg; concentration 2 was 10 times concentration 1) and PCB-1016 and PCB-1260 (10 times concentration 1 of the pesticides). Technical chlordane and toxaphene were fractionated separately. The values given in Tables 33 through 36 are the average recoveries and the standard deviations (numbers in parentheses, from three replicate determinations). These data indicate the accuracy and precision of a method involving silica gel fractionation (procedure given in Section 6.2.1) and analysis by gas chromatography with electron capture detection.

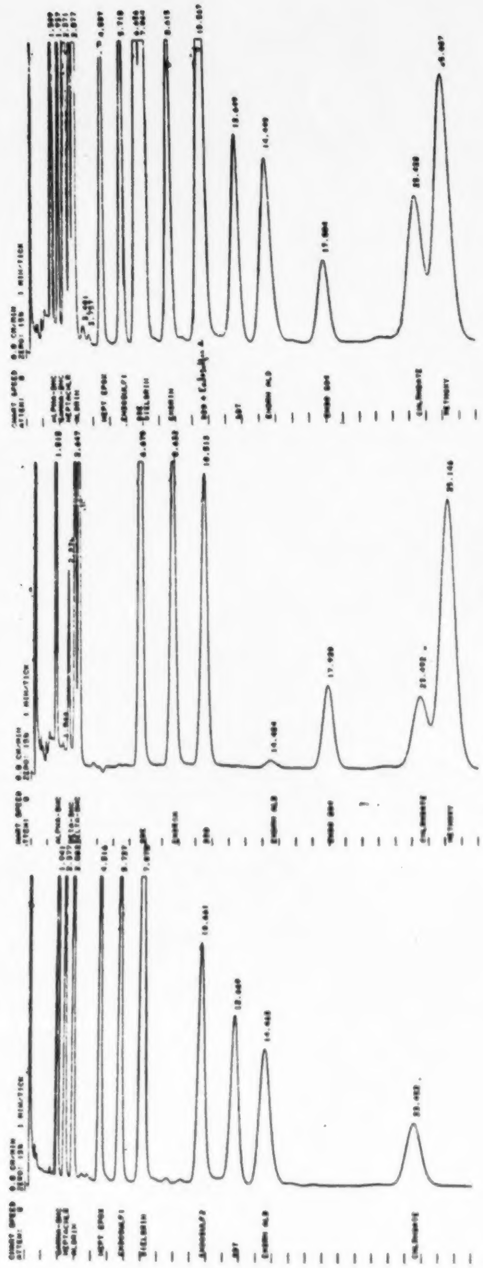

Figure 14. GC/EC chromatograms of the organochlorine pesticide standards A (bottom) and B (middle) and of a composite of A and B (top), obtained on the 1.5-percent OV-17/1.95-percent OV-210 column. Concentrations: standard A, 0.05 to 0.10 ng/μL; standard B, 0.05 to 0.50 ng/μL; standard A and B composite, 0.05 to 0.10 ng/μL; isothermal at 190°C.

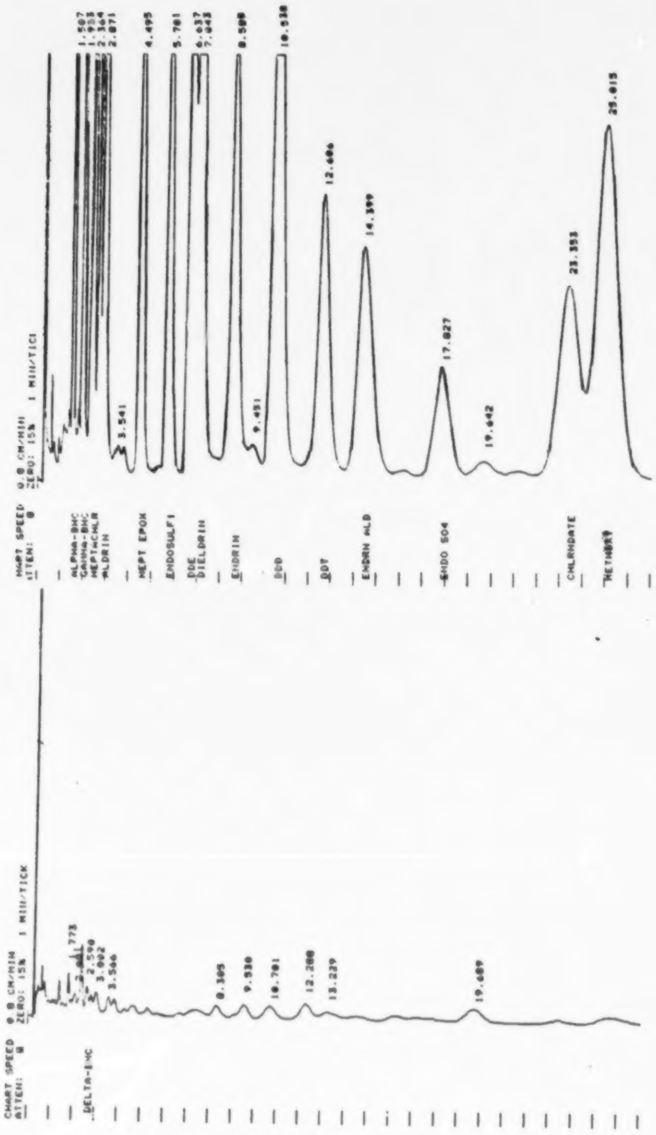

Figure 15. GC/EC chromatograms of a mixture of PCB-1016/1260 at 0.05 ng/µL (bottom) and of a composite of the organochlorine pesticide standards A and B and PCB-1016/1260 at 0.05 ng/µL (top), obtained on the 1.5-percent OV-17/1.95-percent OV-210 column; isothermal at 190°C.

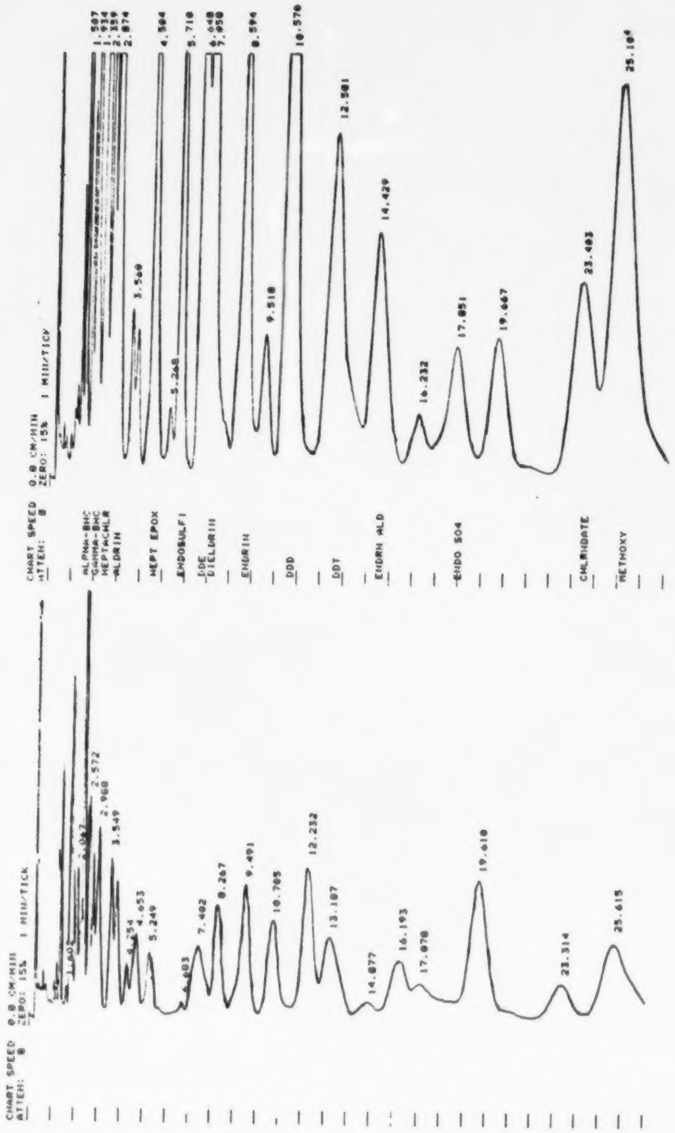

Figure 16. GC/EC chromatograms of a PCB-1016/1260 mixture at 0.5 ng/μL (bottom) and of a composite of organochlorine pesticide standards A and B and PCB-1016/1260 (top) at concentrations of 0.05 to 0.50 ng/μL, respectively. The analyses were performed on the 1.5-percent OV-1 71/1.95-percent OV-210 column; isothermal at 190°C.

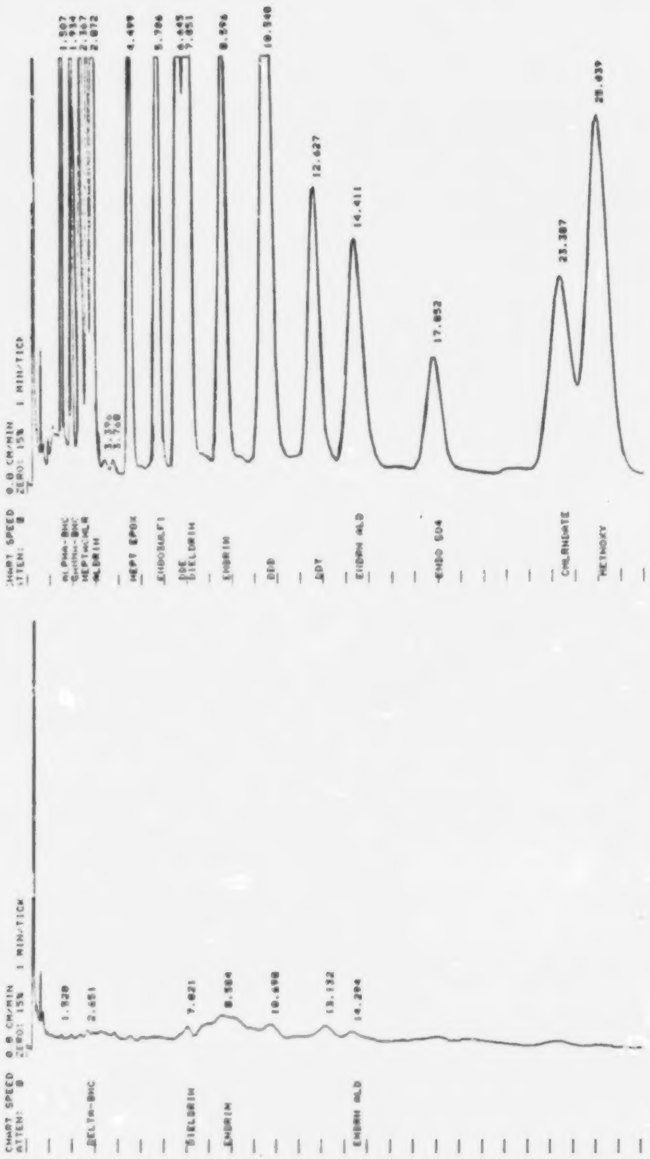

Figure 17. GC/EC chromatograms of the toxaphene standard at 0.10 ng/µL (bottom) and of a composite of organochlorine pesticide standards A and B at 0.05 to 0.50 ng/µL and toxaphene at 0.10 ng/µL (top), obtained on the 1.5-percent OV-17/1.95-percent OV-210 column; isothermal at 190°C.

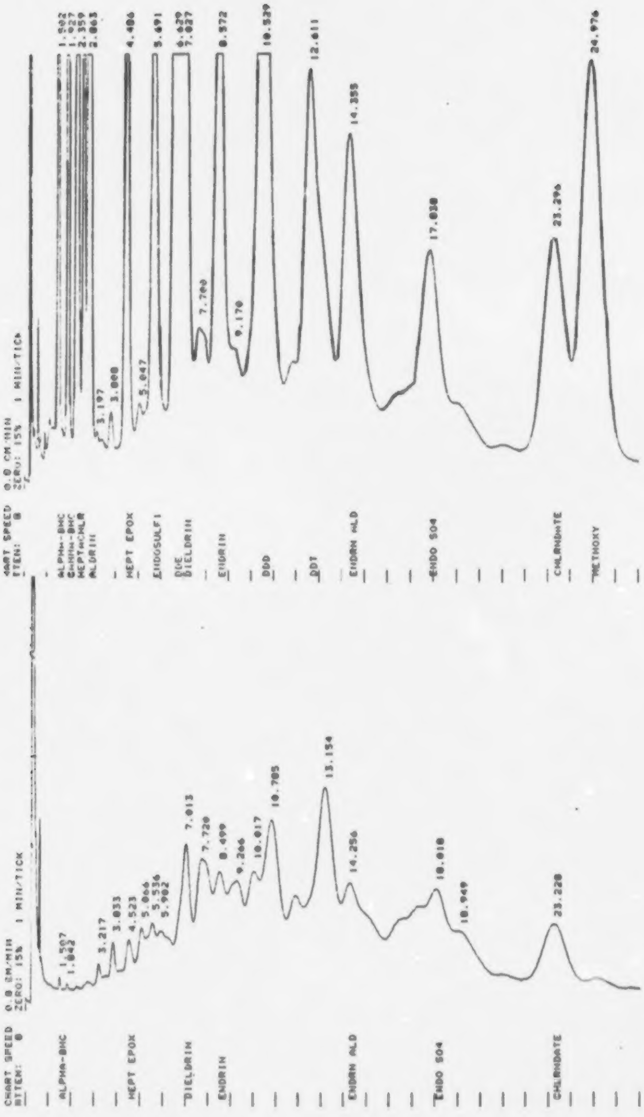

Figure 18. GC/EC chromatograms of the toxaphene standard at 1.0 ng/μL (bottom) and of a composite of organochlorine pesticide standards A and B at 0.05 and 0.50 ng/μL and toxaphene at 1.0 ng/μL (top), obtained on the 1.5-percent OV-17/1.95-percent OV-210 column; isothermal at 190°C.

TABLE 33. ORGANOCHLORINE PESTICIDES AND PCBs AVERAGE RECOVERIES FROM SILICA GEL CHROMATOGRAPHY, DETERMINED ON A OV-17/OV-210 COLUMN[a,b,c,d,e]

	Fraction I		Fraction II		Fraction III		Fraction IV		Total	
	Conc. 1	Conc. 2	Conc. 1	Conc. 2	Conc. 1	Conc. 2	Conc. 1	Conc. 2	Conc. 1	Conc. 2
alpha-BHC					77.8 (1.4)	64.5 (7.7)			77.8 (1.4)	64.5 (7.7)
beta-BHC					74.2 (1.1)	67.8 (8.0)			74.2 (1.1)	67.8 (8.0)
gamma-BHC					86.3 (3.1)	76.9 (8.5)			86.3 (3.1)	76.9 (8.5)
delta-BHC					90.4 (3.3)	78.5 (9.6)			90.4 (3.3)	78.5 (9.6)
Heptachlor	169 (10.0)	175 (12.1)	8.1 (6.4)						178 (15.7)	175 (12.1)
Aldrin	134 (10.5)	136 (4.1)							134 (10.5)	136 (4.1)
Heptachlor epoxide					92.1 (3.2)	79.6 (8.6)			92.1 (3.2)	79.6 (8.6)
Endosulfan I					90.3 (3.2)	82.0 (7.6)			90.3 (3.2)	82.0 (7.6)
4,4'-DDE	82.8 (4.6)	86.9 (5.0)	5.9 (7.6)						88.7 (8.8)	86.9 (5.0)
Dieldrin					91.4 (2.5)	79.2 (8.6)			91.4 (2.5)	79.2 (8.6)
Endrin					75.4 (7.3)	61.6 (10.8)			75.4 (7.3)	61.6 (10.8)
Endosulfan II[f]					91.3 (1.2)	77.8 (9.4)			91.3 (1.2)	77.8 (9.4)
4,4'-DDD[f]					91.3 (1.2)	77.8 (9.4)			91.3 (1.2)	77.8 (9.4)
Kepone							46.6 (3.3)	77.4 (5.1)	46.6 (3.3)	77.4 (5.1)
Endrin aldehyde					77.6 (9.8)	73.4 (8.7)	27.6 (4.6)	33.5 (5.4)	105 (14.1)	107 (7.2)
Endosulfan sulfate					95.2 (5.2)	79.7 (10.7)	7.2 (5.1)		102 (10.2)	79.7 (10.7)
4,4'-DDT			93.0 (11.4)	80.3 (11.6)	16.4 (18.4)	9.0 (14.0)			109 (9.7)	89.3 (23.4)
4,4'-Methoxychlor					109 (0.8)	88.0 (13.9)	6.9 (1.9)	5.3 (2.8)	115 (2.1)	93.3 (12.0)
PCB-1016	91.5 (10.7)	87.1 (14.1)							91.5 (10.7)	87.1 (14.1)
PCB-1260	90.0 (9.6)	97.8 (4.1)							90.0 (9.6)	97.8 (4.1)
Technical chlordane	14.6 (2.3)	16.2 (2.4)	36.4 (1.7)	37.5 (3.4)	42.2 (3.3)	38.8 (6.3)			93.2 (0.9)	93.3 (6.6)
Toxaphene			13.6 (2.1)	13.9 (2.4)	80.0 (4.1)	82.7 (2.7)			93.6 (2.4)	96.3 (1.2)

[a] Eluant composition:
Fraction I - 80 mL hexane.
Fraction II - 50 mL hexane.
Fraction III - 15 mL methylene chloride.
Fraction IV - 50 mL ethyl acetate.

[b] Concentration 1 is 0.5 μg per column for BHCs, heptachlor, aldrin, heptachlor epoxide, endosulfan I; 1.0 μg per column for dieldrin, Kepone, endosulfan II, 4,4'-DDT, endrin aldehyde, 4,4'-DDD, 4,4'-DDE, endrin, and endosulfan sulfate; 5 μg per column for 4,4'-methoxychlor and technical chlordane; 10 μg per column for toxaphene, PCB-1016, and PCB-1260.

[c] For concentration 2 the amounts spiked are 10 times as high as those for concentration 1.

[d] The values given represent the average recoveries of three determinations; the numbers in parentheses are the standard deviations; recovery cut-off point is 5 percent.

[e] Data obtained with standards, as indicated in footnotes b and c, dissolved in 2 mL hexane.

[f] 4,4'-DDD and endosulfan II are not resolved, and an average recovery is given.

TABLE 34. ORGANOCHLORINE, PESTICIDES AND PCBs AVERAGE RECOVERIES FROM SILICA GEL CHROMATOGRAPHY, DETERMINED ON A 3-PERCENT OV-1 COLUMN[a], [b], [c], [d], [e]

	Fraction I		Fraction II		Fraction III		Fraction IV		Total	
	Conc. 1	Conc. 2	Conc. 1	Conc. 2	Conc. 1	Conc. 2	Conc. 1	Conc. 2	Conc. 1	Conc. 2
alpha-BHC					78.1 (2.7)	67.8 (6.7)			78.1 (2.7)	67.8 (6.7)
beta-BHC					102 (2.2)	89.2 (9.9)			102 (2.2)	89.2 (9.9)
gamma-BHC					79.7 (1.7)	77.0 (7.8)			79.7 (1.7)	77.0 (7.8)
delta-BHC					90.1 (6.1)	74.2 (6.6)			90.1 (6.1)	74.2 (6.2)
Heptachlor	94.4 (2.5)	102 (7.6)							94.4 (2.5)	102 (7.6)
Aldrin	110 (9.1)	111 (1.6)							110 (9.1)	111 (1.6)
Heptachlor epoxide					92.7 (9.1)	80.2 (8.3)			92.7 (4.1)	80.2 (8.3)
Endosulfan I					93.0 (4.1)	79.0 (9.5)			93.0 (4.1)	79.0 (9.5)
4,4'-DDE	93.4 (4.6)	96.6 (4.9)	7.9 (8.0)						101.1 (10.9)	96.6 (4.9)
Dieldrin					92.1 (3.2)	78.5 (7.3)			92.1 (3.2)	78.5 (7.3)
Endrin					69.0 (9.6)	58.2 (17.9)			69.0 (9.6)	58.2 (17.9)
Endosulfan II					97.0 (0.9)	80.1 (9.3)			97.0 (0.9)	80.1 (9.3)
4,4'-DDD[f]					50.2 (7.5)	54.6 (9.8)	15.8 (3.2)	21.4 (2.7)	66.1 (10.7)	76.0 (15.2)
Endrin aldehyde[f]					50.2 (7.5)	54.6 (9.8)	15.8 (3.2)	21.4 (2.7)	66.1 (10.7)	76.0 (15.2)
Kepone							57.9 (8.4)	94.8 (8.4)	57.9 (8.4)	94.8 (8.4)
Endosulfan sulfate					93.1 (1.2)	74.8 (7.7)			93.1 (1.2)	74.8 (7.7)
4,4'-DDT			84.7 (10.3)	73.6 (9.4)					84.7 (10.3)	73.6 (9.4)
4,4'-Methoxychlor					115 (1.4)	89.9 (9.2)	7.4 (1.8)	6.3 (3.4)	122 (0.8)	96.2 (7.5)
PCB-1016	87.7 (4.5)	86.4 (9.1)							87.7 (4.5)	86.4 (9.1)
PCB-1260	94.8 (9.3)	99.7 (6.7)							94.8 (9.3)	99.7 (6.7)
Technical chlordane	17.8 (1.4)	18.5 (2.8)	37.8 (1.0)	37.8 (2.6)	38.3 (2.4)	34.0 (4.6)			93.9 (0.8)	90.4 (3.0)
Toxaphene			12.9 (2.4)	12.7 (3.4)	75.9 (8.1)	79.6 (3.7)			88.8 (7.2)	92.1 (0.1)

[a] Eluant composition:
Fraction I - 80 mL hexane.
Fraction II - 50 mL hexane.
Fraction III - 15 mL methylene chloride.
Fraction IV - 50 mL ethyl acetate.

[b] Concentration 1 is 0.5 µg per column for BHCs, heptachlor, aldrin, heptachlor epoxide, endosulfan I; 1.0 µg per column for dieldrin, Kepone, endosulfan II, 4,4'-DDT, endrin aldehyde, 4,4'-DDD, 4,4'-DDE, endrin, and endosulfan sulfate; 5 µg per column for 4,4'-methoxychlor and technical chlordane; 10 µg per column for toxaphene, PCB-1016, and PCB-1260.

[c] For concentration 2 the amounts spiked are 10 times as high as those for concentration 1.

[d] The values given represent the average recoveries of three determinations; the numbers in parentheses are the standard deviations; recovery cut-off point is 5 percent.

[e] Data obtained with standards, as indicated in footnotes b and c, dissolved in 2 mL hexane.

[f] 4,4'-DDD and endrin aldehyde are not resolved, and an average recovery is given.

TABLE 35. AVERAGE RECOVERIES FROM SILICA GEL CHROMATOGRAPHY, DETERMINED ON A 30 m X 0.25 mm ID DB-5 FUSED-SILICA CAPILLARY COLUMN[a,b,c,d,e]

	Fraction I		Fraction II		Fraction III		Fraction IV		Total	
	Conc. 1	Conc. 2	Conc. 1	Conc. 2	Conc. 1	Conc. 2	Conc. 1	Conc. 2	Conc. 1	Conc. 2
alpha-BHC				7.4 (5.5)	82.1 (1.7)	73.6 (8.0)			82.1 (1.7)	81.0 (6.8)
beta-BHC					107 (2.1)	98.1 (12.5)			107 (2.1)	98.1 (12.5)
gamma-BHC					91.3 (3.6)	85.2 (10.7)			91.3 (3.6)	85.2 (10.1)
delta-BHC					92.4 (3.5)	83.5 (10.6)			92.4 (3.5)	83.5 (10.6)
Heptachlor	109 (4.1)	118 (8.7)							109 (4.1)	118 (8.7)
Aldrin	97.5 (5.6)	104 (1.6)							97.5 (5.6)	104 (1.6)
Heptachlor epoxide					94.9 (4.7)	88.2 (10.2)			94.9 (4.7)	88.2 (10.2)
Endosulfan I					95.1 (5.1)	87.0 (10.2)			95.1 (5.1)	87.0 (10.2)
4,4'-DDE	86.1 (5.4)	94.0 (2.8)							86.1 (5.4)	94.0 (2.8)
Dieldrin					96.5 (6.0)	86.8 (10.6)			96.5 (6.0)	86.8 (10.6)
Endrin					84.7 (10.5)	71.0 (12.3)			84.7 (10.5)	71.0 (12.3)
Endosulfan II					96.8 (4.4)	85.6 (10.4)			96.8 (4.4)	85.6 (10.4)
4,4'-DDD					102 (4.6)	92.2 (10.2)			102 (4.6)	92.2 (10.2)
Endrin aldehyde					80.8 (1.9)	75.8 (9.5)	29.4 (4.2)	33.2 (4.7)	110 (3.0)	109 (5.8)
Kepone							40.7 (7.9)	73.0 (12.0)	40.7 (7.9)	73.0 (12.0)
Endosulfan sulfate					93.0 (4.9)	82.2 (9.2)			93.0 (4.9)	82.2 (9.2)
4,4'-DDT			86.2 (13.4)	72.7 (9.1)	14.7 (18.7)				101 (5.3)	72.7 (9.1)
4,4'-Methoxychlor					98.8 (9.9)	82.3 (10.7)	9.0 (4.8)	5.7 (3.0)	108 (10.0)	88.0 (5.8)
PCB-1016	86.1 (4.0)	86.8 (6.1)							86.1 (4.0)	86.8 (6.1)
PCB-1260	90.9 (4.1)	95.3 (5.0)							90.9 (4.1)	95.3 (5.0)
Technical chlordane	13.9 (5.5)	22.4 (5.3)	19.3 (6.8)	38.7 (3.6)	29.2 (5.0)	37.3 (5.1)			62.4 (3.3)	98.4 (1.9)
Toxaphene			14.9 (2.4)	16.7 (1.4)	73.1 (9.4)	84.2 (10.7)			88.0 (12.0)	101 (10.1)

[a] Eluant composition:
Fraction I - 80 mL hexane.
Fraction II - 50 mL hexane.
Fraction III - 15 mL methylene chloride.
Fraction IV - 50 mL ethyl acetate.

[b] Concentration 1 is 0.5 µg per column for BHCs, heptachlor, aldrin, heptachlor epoxide, endosulfan I; 1.0 µg per column for dieldrin, Kepone, endosulfan II, 4,4'-DDT, endrin aldehyde, 4,4'-DDD, 4,4'-DDE, endrin, and endosulfan sulfate; 5 µg per column for 4,4'-methoxychlor and technical chlordane; 10 µg per column for toxaphene, PCB-1016, and PCB-1260.

[c] For concentration 2 the amounts spiked are 10 times as high as those for concentration 1.

[d] The values given represent the average recoveries of three determinations; the numbers in parentheses are the standard deviations; recovery cut-off point is 5 percent.

[e] Data obtained with standards, as indicated in footnotes b and c, dissolved in 2 mL hexane.

TABLE 36. AVERAGE RECOVERIES FROM SILICA GEL CHROMATOGRAPHY, DETERMINED ON AN SPB-608 FUSED-SILICA CAPILLARY COLUMN[a,b,c,d,e]

	Fraction I		Fraction II		Fraction III		Fraction IV		Total	
	Conc. 1	Conc. 2	Conc. 1	Conc. 2	Conc. 1	Conc. 2	Conc. 1	Conc. 2	Conc. 1	Conc. 2
alpha-BHC					85.7 (1.7)	70.8 (7.4)			85.7 (1.7)	70.8 (7.4)
beta-BHC					109 (1.0)	93.7 (11.4)			109 (1.0)	93.7 (11.4)
gamma-BHC					86.6 (4.0)	72.9 (8.1)			86.6 (4.0)	72.9 (8.1)
delta-BHC					93.1 (1.3)	78.7 (8.9)			93.1 (1.3)	78.7 (8.9)
Heptachlor	110 (4.1)	116 (6.0)	9.1 (6.9)						119 (7.1)	116 (6.0)
Aldrin	97.8 (7.8)	102 (2.8)							97.8 (7.8)	102 (2.8)
Heptachlor epoxide					86.0 (2.8)	75.4 (7.6)			86.0 (2.8)	75.4 (7.6)
Endosulfan I					86.3 (2.5)	76.0 (8.0)			86.3 (2.5)	76.0 (8.0)
4,4'-DDE	87.3 (5.1)	92.5 (4.2)	9.4 (10.1)						96.7 (9.5)	92.5 (4.2)
Dieldrin					83.9 (1.7)	73.4 (6.8)			83.9 (1.7)	73.4 (6.8)
Endrin					80.8 (6.1)	64.7 (10.2)			80.8 (6.1)	64.7 (10.2)
Endosulfan II					84.5 (0.2)	73.2 (7.6)			84.5 (0.2)	73.2 (7.6)
4,4'-DDD					95.8 (2.8)	81.6 (7.0)			95.8 (2.8)	81.6 (7.0)
Endrin aldehyde					70.1 (11.2)	66.6 (7.1)	22.0 (4.8)	28.6 (4.7)	92.1 (14.6)	95.2 (4.7)
Endosulfan sulfate					95.4 (1.9)	80.6 (8.5)			95.4 (1.9)	80.6 (8.5)
4,4'-DDT			76.2 (5.4)	64.4 (9.8)					76.2 (5.4)	64.4 (9.8)
4,4'-Methoxychlor					83.9 (1.8)	71.0 (6.8)	6.8 (0.8)	6.1 (2.8)	90.7 (1.0)	77.1 (5.0)
PCB-1016	86.6 (3.2)	76.7 (7.0)							86.6 (3.2)	76.7 (7.0)
PCB-1260	87.9 (5.3)	93.1 (7.4)							87.9 (5.3)	93.1 (7.4)
Technical chlordane	24.8 (2.1)	24.1 (3.1)	47.9 (10.2)	42.8 (9.3)	40.2 (1.7)	31.7 (3.6)			113 (14.0)	98.6 (9.2)
Toxaphene			14.5 (1.8)	16.6 (0.6)	77.9 (2.4)	83.7 (0.7)			92.4 (4.1)	100 (0.7)

[a] Eluant composition:
Fraction I - 80 mL hexane.
Fraction II - 50 mL hexane.
Fraction III - 15 mL methylene chloride.
Fraction IV - 50 mL ethyl acetate.

[b] Concentration 1 is 0.5 µg per column for BHCs, heptachlor, aldrin, heptachlor epoxide, endosulfan I; 1.0 µg per column for dieldrin, Kepone, endosulfan II, 4,4'-DDT, endrin aldehyde, 4,4'-DDD, 4,4'-DDE, endrin, and endosulfan sulfate; 5 µg per column for 4,4'-methoxychlor and technical chlordane; 10 µg per column for toxaphene, PCB-1016, and PCB-1260.

[c] For concentration 2 the amounts spiked are 10 times as high as those for concentration 1.

[d] The values given represent the average recoveries of three determinations; the numbers in parentheses are the standard deviations; recovery cut-off point is 5 percent.

[e] Data obtained with standards, as indicated in footnotes b and c, dissolved in 2 mL hexane.

6.4 RECOMMENDATIONS FOR METHOD REVISION

Upon completion of the various method improvement studies described in Sections 6.1, 6.2, and 6.3, the following recommendations were made for consideration in revising Method 8080:

- Methods 3510 and 3520 give comparable accuracies when evaluated with spiked distilled water samples. Except for Kepone and endosulfan II, all compounds yielded recoveries >70 percent with either method. Method precision was better for Method 3520. However, when the liquid samples are highly contaminated and sample portions of less than 100 mL are extracted, Method 3520 is not desirable with the equipment specified in the current method since additional organics-free water has to be added which increases the possibility of introducing contamination.
- Methods 3540 and 3550, recommended for the extraction of solid matrices, give comparable accuracies. Method 3550 appears to give better precision than Method 3540. The change in the composition of the extraction solvent was found to affect the performance of Method 3550 slightly, however, it did not justify a change in the Method 3550 protocol.
- The silica gel fractionation procedure separates the PCBs from most of the organochlorine pesticides. The distribution patterns of the organochlorine pesticides in the four fractions were reproducible. Components found to elute in Fraction I include heptachlor, aldrin, 4,4'-DDE, 4,4'-DDT, and the PCBs. Almost all other organochlorine pesticides elute in Fraction III. A few compounds, 4,4'-DDD, beta-BHC, and gamma-chlordane, were distributed between two or three fractions. This fractionation procedure should be further evaluated with additional real samples to obtain method performance information.
- Packed column analysis should be replaced with the capillary column analysis. Two capillary columns have been selected for further evaluation.

SECTION 7

REVISED METHOD 8080 -- EVALUATION STUDIES

The performance of the revised Method 8080 was evaluated to determine the reproducibility and efficiency of the silica gel fractionation, the precision of the identification and the measurement of the gas chromatographic technique, and the minimum detectable levels for the organochlorine pesticides and PCBs. The subsections address the reproducibility of the gas chromatographic analysis, the method precision and accuracy, and the method detection limits.

7.1 REPRODUCIBILITY STUDY OF THE GAS CHROMATOGRAPHIC ANALYSIS

To establish the precision of the gas chromatographic method, ten replicate injections of a standard containing the organochlorine pesticides were performed by using an autosampler. Separately, another set of ten replicate injections were performed of a mixture of PCB-1016 and PCB-1260 and of toxaphene. The results are presented as the average response factors of the ten replicates and the relative standard deviations (RSDs), and as the average retention times of the ten replicates and the RSDs (Tables 37 and 38).

The reproducibility of the injection technique was better than 10 percent for the early eluting compounds, and was between 10 and 16 percent for 4,4'-DDE, 4,4'-DDD, 4,4'-DDT, endosulfan sulfate, endrin aldehyde, and 4,4'-methoxychlor. The reproducibility of the retention times was excellent (<0.15 percent) for the organochlorine pesticides.

In the case of the PCBs, the peak areas were summed for all peaks, and a response factor was determined from the total response and from the amount injected (Table 39). Six major components that represent the mixture were selected and were used to calculate the relative standard deviation of the ten replicate measurements (Table 40). The reproducibility of the injection, when the sum of all peaks was used in the calculations, was better than 15 percent. As expected, the reproducibility was better for the early eluting peaks but degraded for the late-eluting components of the PCB mixture. The reproducibility of the retention time was also excellent for the two PCB mixtures tested (Table 41). This indicates that the identification of the organochlorine pesticides and PCBs by retention time match is reliable for screening purposes.

TABLE 37. REPRODUCIBILITY OF THE RESPONSE FACTORS OF THE ORGANOCHLORINE PESTICIDES FOR 10 CONSECUTIVE REPLICATE INJECTIONS[a]

Compounds	Response factor x 10^{-6}											
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	Average	RSD (percent)
alpha-BHC	2.76	2.87	2.97	2.92	2.99	2.83	2.88	2.79	2.97	2.89	2.88	2.5
beta-BHC	0.94	0.86	0.99	0.90	0.99	0.84	0.92	0.81	0.99	0.92	0.92	6.9
gamma-BHC	2.54	2.54	2.70	2.60	2.67	2.50	2.58	2.47	2.65	2.58	2.58	2.9
delta-BHC	2.40	2.29	2.56	2.41	2.57	2.27	2.45	2.23	2.57	2.44	2.42	5.2
Heptachlor	2.19	2.11	2.33	2.20	2.34	2.07	2.24	2.04	2.34	2.25	2.21	5.0
Aldrin	2.22	2.10	2.36	2.23	2.39	2.08	2.28	2.04	2.39	2.29	2.23	5.8
Heptachlor epoxide	2.03	1.88	2.23	2.07	2.23	1.90	2.17	1.85	2.27	2.13	2.08	7.5
gamma-Chlordane	2.05	1.85	2.20	2.01	2.21	1.86	2.13	1.82	2.24	2.13	2.05	8.0
Endosulfan I	1.90	1.67	2.07	1.82	2.01	1.67	1.93	1.63	2.05	1.93	1.87	8.7
4,4'-DDE + Dieldrin	3.29	2.92	3.50	3.12	3.50	2.88	3.34	2.81	3.55	3.36	3.23	8.6
Endrin	1.28	1.01	1.32	1.08	1.26	9.10	1.06	8.76	1.25	1.16	1.12	14.1
Endosulfan II	1.75	1.50	1.86	1.63	1.87	1.49	1.78	1.45	1.91	1.79	1.70	10.1
4,4'-DDD	1.50	1.23	1.60	1.38	1.60	1.23	1.47	1.17	1.64	1.52	1.43	12.0
Endrin aldehyde	1.35	1.10	1.46	1.28	1.42	1.15	1.36	1.05	1.53	1.42	1.32	12.4
Endosulfan sulfate	1.51	1.20	1.59	1.35	1.58	1.20	1.46	1.16	1.63	1.52	1.42	12.6
4,4'-DDT	1.47	1.15	1.50	1.29	1.50	1.13	1.37	1.09	1.53	1.43	1.35	12.5
4,4'-Methoxychlor	0.57	0.42	0.61	0.49	0.57	0.41	0.47	0.39	0.59	0.55	0.51	16.1

[a] The amount injected is 100 pg per component; the response factor is defined as peak area divided by the amount injected.

[b] The analyses were performed on a 30 m x 0.25 mm ID DB-5 fused-silica capillary column; temperature program: 160°C (2 min hold) to 270°C (1 min hold) at 5°C/min; carrier gas: nitrogen at 20 psi.

TABLE 38. REPRODUCIBILITY OF THE RETENTION TIMES OF THE ORGANOCHLORINE PESTICIDES FOR 10 CONSECUTIVE REPLICATE INJECTIONS[a]

Compounds	Retention time (min.)											RSD (percent)
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	Average	
alpha-BHC	6.515	6.511	6.506	6.500	6.487	6.520	6.512	6.501	6.501	6.498	6.505	0.149
beta-BHC	7.274	7.261	7.261	7.260	7.243	7.267	7.265	7.267	7.254	7.253	7.260	0.120
gamma-BHC	7.499	7.475	7.476	7.482	7.464	7.495	7.496	7.486	7.473	7.481	7.482	0.151
delta-BHC	8.206	8.200	8.197	8.195	8.173	8.208	8.208	8.195	8.193	8.185	8.196	0.133
Heptachlor	9.893	9.880	9.875	9.882	9.865	9.889	9.886	9.880	9.870	9.877	9.880	0.086
Aldrin	11.099	11.093	11.085	11.087	11.071	11.095	11.102	11.095	11.083	11.082	11.089	0.084
Heptachlor epoxide	12.531	12.521	12.517	12.516	12.503	12.525	12.529	12.524	12.507	12.521	12.519	0.071
gamma-Chlordane	13.399	13.398	13.381	13.381	13.367	13.390	13.398	13.393	13.369	13.382	13.386	0.087
Endosulfan I	13.849	13.831	13.833	13.829	13.812	13.843	13.834	13.834	13.820	13.832	13.832	0.075
4,4'-DDE + Dieldrin	14.799	14.794	14.790	14.792	14.776	14.801	14.801	14.800	14.784	14.787	14.792	0.056
Endrin	15.590	15.582	15.581	15.580	15.571	15.586	15.589	15.583	15.569	15.579	15.581	0.044
Endosulfan II	15.933	15.923	15.922	15.920	15.908	15.922	15.924	15.923	15.917	15.916	15.921	0.040
4,4'-DDD	16.341	16.329	16.326	16.333	16.313	16.333	16.338	16.333	16.320	16.323	16.329	0.052
Endrin aldehyde	16.634	16.638	16.631	16.632	16.618	16.633	16.642	16.636	16.623	16.628	16.631	0.042
Endosulfan sulfate	17.528	17.523	17.513	17.523	17.503	17.522	17.523	17.524	17.508	17.513	17.518	0.047
4,4'-DDT	17.729	17.728	17.722	17.726	17.711	17.730	17.739	17.735	17.721	17.722	17.726	0.045
4,4'-Methoxychlor	19.924	19.917	19.918	19.918	19.900	19.916	19.922	19.917	19.907	19.913	19.915	0.035

[a]The analyses were performed on a 30 m x 0.25 mm ID DB-5 fused-silica capillary column; temperature program: 160°C (2 min hold) to 270°C (1 min hold) at 5°C/min; carrier gas: nitrogen at 20 psi.

TABLE 39. REPRODUCIBILITY OF THE RESPONSE FACTORS FOR 10 CONSECUTIVE INJECTIONS OF PCB-1016/1260 STANDARDS[a,b]

	Response factor					
Compounds	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
PCB-1016/1260	1.868×10^6	1.514×10^6	1.310×10^6	1.630×10^6	1.283×10^6	1.858×10^6

	Response factor					
Compounds	No. 7	No. 8	No. 9	No. 10	Average	RSD (percent)
PCB-1016/1260	1.525 x 106	1.711 x 106	1.805 x 106	1.284 x 106	1.579 x 106	14.7

[a] The amount injected is 100 pg per component; the response factor is defined as peak area divided by the amount injected.

[b] The analyses were performed on a 30 m x 0.25 mm ID DB-5 fused-silica capillary column; temperature program: 160°C (2 min hold) to 270°C (1 min hold) at 5°C/min; carrier gas: nitrogen at 20 psi.

TABLE 40. REPRODUCIBILITY OF AREA RESPONSE FOR 10 CONSECUTIVE INJECTIONS OF PCB-1016/1260 STANDARDS (SIX MAJOR COMPONENTS)[a]

Retention time (min.)	Peak area											
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	Average	RSD (percent)
9.327	107856	100454	112331	99213	121480	108640	117956	115803	93180	115883	109280	8.4
15.936	64705	53292	69306	52933	80229	65305	72680	75767	54488	75623	66433	15.1
18.520	52137	42329	56945	41930	67907	54236	60270	63450	44894	63106	54720	17.0
19.371	44206	36759	50050	35770	59016	46733	53064	56815	38747	56027	47719	18.1
20.402	106234	90652	126169	86008	149645	114682	133804	142659	95441	144975	119027	19.9
21.467	48269	41379	57653	38885	68117	51297	60409	65657	42936	68507	54310	20.8

[a] Analyses were performed on a 30 m x 0.25 mm ID DB-5 fused-silica capillary column; temperature program: 160°C (2 min hold) to 270°C (1 min hold) at 5°C/min; carrier gas: nitrogen at 20 psi.

TABLE 41. REPRODUCIBILITY OF RETENTION TIMES OF SIX MAJOR COMPONENTS IN A PCB-1016/1260 MIXTURE FOR 10 CONSECUTIVE INJECTIONS[a]

	Retention time (min)											
Peak No.	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	Average	RSD (percent)
1	9.333	9.327	9.340	9.35	9.335	9.335	9.312	9.321	9.346	9.335	9.32	0.104
2	15.963	15.962	15.966	15.968	15.969	15.963	15.948	15.958	15.967	15.956	15.962	0.040
3	18.523	18.520	18.528	18.529	18.528	18.525	18.504	18.522	18.518	18.514	18.521	0.042
4	19.377	19.371	19.380	19.381	19.379	19.378	19.356	19.378	19.372	19.367	19.374	0.040
5	20.402	20.393	20.405	20.407	20.403	20.407	20.382	20.397	20.392	20.390	20.398	0.041
6	21.474	21.467	21.472	21.482	21.477	21.481	21.458	21.471	21.466	21.465	21.471	0.035

[a] Analyses were performed on a 30 m x 0.25 mm ID DB-5 fused-silica capillary column; temperature program: 160°C (2 min hold) to 270°C (1 min hold) at 5°C/min; carrier gas: nitrogen at 20 psi.

7.2 METHOD PRECISION AND ACCURACY

Experimental Section

Sample Collection: Liquid waste No. 1 was collected from a pesticide waste storage facility at a California agricultural field station. This waste contains high concentrations of organics (total organic carbon 520 mg/L; total organic halogen 30 mg/L) and was briefly described in Section 5. The standard reference material was NBS SRM 1645. According to the NBS, the material was dredged from the bottom of the Indiana Harbor Canal near Gary, Indiana. The material was screened to remove foreign objects, freeze-dried, and sieved (particle size <180 µm). The material was sterilized by radiation to minimize biological activity. A gross characterization done by NBS was also given in Section 5.

Sandy loam was obtained from Soils Incorporated, Puyallup, Washington; the following physicochemical characteristics were determined: pH 5.9 to 6.0; 89 percent sand; 7 percent silt; 4 percent clay; cation exchange capacity 7.5 meq/100g; total organic carbon content 1290 ± 185 mg/Kg.

Reagents. Stock solutions of 4,4'-methoxychlor (5000 ng/µL in methylene chloride), gamma-chlordane (5000 ng/µL in hexane) and a composite solution of aldrin, alpha-BHC, beta-BHC, gamma-BHC, delta-BHC, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, dieldrin, endosulfan I, endosulfan II, endosulfan sulfate, endrin, endrin aldehyde, heptachlor, and heptachlor epoxide, at 2,000 ng/µL in toluene-hexane (1:1), were obtained from the U.S. EPA Quality Assurance Materials Bank, Research Triangle Park, North Carolina. Neat standards of Kepone, PCB-1016, and PCB-1260 were obtained from the U.S. EPA Pesticides and Industrial Chemicals Repository, Research Triangle Park, North Carolina, and were used to prepare stock solutions of Kepone at 1000 ng/µL each in benzene and of PCB-1016 and PCB-1260 together at 2,500 ng/µL in hexane. These individual stock solutions and the composite solutions were further diluted with hexane to obtain the spiking solutions and the analytical standards for instrument calibration.

Sample Extract Preparation. The liquid waste No. 1 (300 mL) was extracted in a separatory funnel at neutral pH with 60 mL methylene chloride. The extraction was repeated two times; fresh aliquots of methylene chloride were used each time. The extracts were combined, were dried through a column of anhydrous sodium sulfate, and were then concentrated in a Kuderna-Danish evaporator. The solvent was exchanged to hexane and concentrated to 30 mL. The 30-mL concentrated extract was split as follows: a 2-mL aliquot was used for background analysis; three aliquots of 6 mL each were spiked with 1,500 ng (concentration 1), 3,000 ng (concentration 2), and 15,000 ng (concentration 3) of the organochlorine pesticides, and 15,000 ng (concentration 1), 30,000 ng (concentration 2), and 150,000 ng (concentration 3) of PCB-1016 and of PCB-1260, respectively.

A 30-g portion of the NBS SRM-1645 or of the sandy loam was extracted with 60-mL portions of hexane-acetone (1:1) by using a sonicator model W-375 from Heat Systems-Ultrasonics, Inc. The extraction was performed three times for 3 min each time with fresh portions of hexane-acetone. The extracts were combined, were concentrated to 30 mL in a Kuderna-Danish evaporator, and were split and spiked as described above for liquid waste No. 1.

Silica Gel Fractionation. All extracts were subjected to silica gel chromatography on silica deactivated with 3.3 percent water (Davidson grade 923, 100/200 mesh, Supelco Inc.). The elution patterns and the recoveries of the 19 organochlorine pesticides and of PCB-1016 and PCB-1260 were determined in triplicate at three concentrations (1,500 ng, 3,000 ng, 15,000 ng for each compound per column) corresponding to the spiking levels in the liquid waste and in the solid samples. PCBs were spiked at concentrations ten times as high as those of the pesticides. Four fractions were collected: Fraction I eluted with 80 mL hexane; Fraction II eluted with 50 mL hexane; Fraction III eluted with 15 mL methylene chloride; and Fraction IV eluted with 50 mL ethyl acetate. The Fraction III solvent was exchanged to hexane, and the Fraction IV solvent was exchanged to 2 percent methanol in benzene prior to analysis. The final volume of each fraction was 10 mL.

Gas Chromatography/Electron Capture Detection. A Varian Vista 6000 gas chromatograph equipped with a split/splitless injector and fused-silica capillary column (30 m x 0.25 mm ID; film thickness 0.25 µm) DB-5 (J&W Scientific, Rancho Cordova, California) was used for analyzing all sample extracts and silica gel fractions. The oven was held at 100°C for 2 min during sample injection, the temperature was then programmed to increase at 15°C/min to 160°C and at 5°C/min to 270°C. The injector and detector temperatures were set at 225°C and at 300°C, respectively. Quantification was performed by external standard calibration. The PCBs were identified by visual comparison of the sample extract chromatograms with the PCB standard chromatograms. Five to six gas chromatographic peaks were selected to quantify each PCB mixture, and the response factors were determined by the analysis of standards containing known concentrations of each PCB mixture.

RESULTS AND DISCUSSION

The accuracy and the precision data are given in Tables 42 through 46 as the average percent recovery ± one standard deviation and as the relative standard deviation of the average recovery (number of determinations is three). The recovery was calculated as the ratio of the amount of a given compound detected to the amount added to the sample extract prior to silica gel fractionation. The amount of compound detected is computed from the detector response relative to the standard. Precision is expressed as percent relative standard deviation.

From these data we conclude that the four-fraction silica gel fractionation allows separation of the organochlorine pesticides from PCBs, with the exception of heptachlor, aldrin, gamma-chlordane, 4,4'-DDE, and 4,4'-DDT. The silica gel procedure is tedious and does account for a major part of the analysis time. However, we have demonstrated that the method precision is better than ±20 percent for all compounds, and the accuracy is greater than 60 percent when standards are processed through the silica gel procedure. Furthermore, the use of a capillary column for gas chromatographic

TABLE 42. ELUTION PATTERNS AND AVERAGE RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs AFTER SILICA GEL CHROMATOGRAPHY (STANDARDS ONLY)

Compound	Average ± SD (RSD) at Concentration 1[a]				
	Fraction I hexane (80 mL)	Fraction II hexane (50 mL)	Fraction III methylene chloride (15 mL)	Fraction IV ethyl acetate (50 mL)	Total recovery
alpha-BHC		59 ± 15 (25)	25 ± 9.2 (37)		83 ± 16 (19)
beta-BHC			86 ± 15 (17)		86 ± 15 (17)
gamma-BHC			85 ± 15 (18)		85 ± 15 (18)
delta-BHC			87 ± 12 (14)		87 ± 12 (14)
Heptachlor	79 ± 11 (13)				79 ± 11 (13)
Aldrin	88 ± 11 (13)				88 ± 11 (13)
Heptachlor epoxide			94 ± 6.7 (7.1)		94 ± 6.7 (7.1)
gamma-Chlordane	78 ± 13 (17)	15 ± 4.7 (31)			94 ± 13 (14)
Endosulfan I			89 ± 12 (14)		89 ± 12 (14)
4,4'-DDE	92 ± 12 (14)				92 ± 13 (14)
Dieldrin			89 ± 12 (14)		89 ± 12 (14)
Endrin			66 ± 11 (17)		66 ± 11 (17)
Endosulfan II			86 ± 7.9 (9.2)		86 ± 7.9 (9.2)
4,4'-DDD		39 ± 15 (38)	50 ± 11 (22)		89 ± 12 (14)
Endrin aldehyde			83 ± 8.3 (10)		83 ± 8.3 (10)
Kepone				0	0
Endosulfan sulfate			91 ± 5.2 (5.7)		91 ± 5.2 (5.7)
4,4'-DDT	74 ± 19 (26)				74 ± 19 (26)
4,4'-Methoxychlor			98 ± 2.6 (2.7)		98 ± 2.6 (2.7)
PCB-1016	94 ± 14 (15)				94 ± 14 (15)
PCB-1260	92 ± 12 (13)				92 ± 12 (13)

[a] The values given represent the average percent recoveries of the organochlorine pesticides and PCBs from five replicate determinations ± one standard deviation; the numbers in parentheses are the relative standard deviations.

TABLE 42. ELUTION PATTERNS AND AVERAGE RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs AFTER SILICA GEL CHROMATOGRAPHY (STANDARDS ONLY) (CONTINUED)

Compound	Ave ± SD (RSD) at Concentration 2[a]				
	Fraction I hexane (80 mL)	Fraction II hexane (50 mL)	Fraction III methylene chloride (15 mL)	Fraction IV ethyl acetate (50 mL)	Total recovery
alpha-BHC		64 ± 6.0 (9.5)	41 ± 5.9 (14)		106 ± 6.8 (6.4)
beta-BHC			110 ± 10 (9.5)		110 ± 10 (9.5)
gamma-BHC			108 ± 11 (10)		108 ± 11 (10)
delta-BHC			109 ± 12 (11)		109 ± 12 (11)
Heptachlor	94 ± 9.5 (10)				94 ± 9.5 (10)
Aldrin	107 ± 9.5 (8.9)				107 ± 9.5 (8.9)
Heptachlor epoxide			109 ± 14 (13)		109 ± 14 (13)
gamma-Chlordane	86 ± 8.4 (9.4)	23 ± 3.2 (14)			110 ± 11 (10)
Endosulfan I			108 ± 13 (12)		108 ± 13 (12)
4,4'-DDE	107 ± 15 (14)				107 ± 15 (14)
Dieldrin			112 ± 13 (12)		112 ± 13 (12)
Endrin			65 ± 10 (16)		65 ± 10 (16)
Endosulfan II			111 ± 14 (13)		111 ± 14 (13)
4,4'-DDD		38 ± 3.5 (9.2)	71 ± 13 (18)		110 ± 9.8 (8.9)
Endrin aldehyde			102 ± 19 (19)		102 ± 19 (19)
Kepone				0	0
Endosulfan sulfate			112 ± 21 (19)		112 ± 21 (19)
4,4'-DDT	88 ± 13 (15)				88 ± 13 (15)
4,4'-Methoxychlor			104 ± 18 (17)		104 ± 18 (17)
PCB-1016	93 ± 6.5 (7.0)				93 ± 6.5 (7.0)
PCB-1260	87 ± 15 (17)				87 ± 15 (17)

[a] The values given represent the average percent recoveries from three replicate determinations ± one standard deviation; the numbers in parentheses are the relative standard deviations.

TABLE 42. ELUTION PATTERNS AND AVERAGE RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs AFTER SILICA GEL CHROMATOGRAPHY (STANDARDS ONLY) (CONCLUDED)

	Average ± SD (RSD) at Concentration 3[a]				
Compound	Fraction I hexane (80 mL)	Fraction II hexane (50 mL)	Fraction III methylene chloride (15 mL)	Fraction IV ethyl acetate (50 mL)	Total recovery
alpha-BHC		45 ± 12 (26)	47 ± 10 (22)		91 ± 4.6 (5.1)
beta-BHC			98 ± 2.0 (2.1)		98 ± 2.0 (2.1)
gamma-BHC			99 ± 2.3 (2.3)		99 ± 2.3 (2.3)
delta-BHC			97 ± 1.6 (1.6)		97 ± 1.6 (1.6)
Heptachlor	83 ± 6.6 (7.9)				83 ± 6.6 (7.9)
Aldrin	89 ± 4.2 (4.7)				89 ± 4.2 (4.7)
Heptachlor epoxide			100 ± 23 (23)		100 ± 23 (23)
gamma-Chlordane	70 ± 7.7 (11)	20 ± 6.8 (34)			91 ± 1.2 (1.3)
Endosulfan I			99 ± 2.3 (2.3)		99 ± 2.3 (23)
4,4'-DDE	89 ± 4.5 (5.0)				89 ± 4.5 (5.0)
Dieldrin			102 ± 1.5 (1.5)		102 ± 1.5 (1.5)
Endrin			64 ± 8.3 (13)		64 ± 8.3 (13)
Endosulfan II			101 ± 0.6 (0.6)		101 ± 0.6 (0.6)
4,4'-DDD		24 ± 10 (42)	73 ± 8.8 (12)		97 ± 1.7 (1.8)
Endrin aldehyde			95 ± 3.0 (3.2)		95 ± 3.0 (3.2)
Kepone				58 ± 25 (44)	58 ± 25 (44)
Endosulfan sulfate			104 ± 2.5 (2.4)		104 ± 2.5 (2.4)
4,4'-DDT	73 ± 5.8 (8.0)				73 ± 5.8 (8.0)
4,4'-Methoxychlor			104 ± 3.2 (3.1)		104 ± 3.2 (3.1)
PCB-1016	93 ± 2.0 (2.2)				93 ± 2.0 (2.2)
PCB-1260	78 ± 4.0 (5.1)				78 ± 4.0 (5.1)

[a] The values given represent the average percent recoveries from three replicate determinations ± one standard deviation; the numbers in parentheses are the relative standard deviations.

TABLE 43. ELUTION PATTERNS AND AVERAGE RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs AFTER SILICA GEL CHROMATOGRAPHY (LIQUID WASTE NO. 1 EXTRACT)

Compound	Average ± SD (RSD) at Concentration 1[a]				
	Fraction I hexane (80 mL)	Fraction II hexane (50 mL)	Fraction III methylene chloride (15 mL)	Fraction IV ethyl acetate (50 mL)	Total recovery
alpha-BHC		58 ± 13 (22)	39 ± 5.5 (14)		96 ± 7.0 (7.3)
beta-BHC			92 ± 10 (11)		92 ± 10 (11)
gamma-BHC			91 ± 10 (11)		91 ± 10 (11)
delta-BHC					i
Heptachlor	87 ± 13 (15)				87 ± 13 (15)
Aldrin	96 ± 8.9 (9.3)				96 ± 8.9 (9.3)
Heptachlor epoxide			111 ± 17 (15)		111 ± 17 (15)
gamma-Chlordane	83 ± 12 (14)	17 ± 6.3 (37)			100 ± 8.5 (8.5)
Endosulfan I			95 ± 6.5 (6.8)		95 ± 6.5 (6.8)
4,4'-DDE	119 ± 11 (8.9)				119 ± 11 (8.9)
Dieldrin			88 ± 3.2 (3.6)		88 ± 3.2 (3.6)
Endrin			101 ± 5.9 (5.8)		101 ± 5.9 (5.8)
Endosulfan II			i		i
4,4'-DDD		32 ± 13 (40)	i		i
Endrin aldehyde			i		i
Kepone				0	0
Endosulfan sulfate			132 ± 17 (13)		132 ± 17 (13)
4,4'-DDT	101 ± 23 (23)				101 ± 23 (23)
4,4'-Methoxychlor			49 ± 14 (29)		49 ± 14 (29)
PCB-1016	114 ± 6.0 (5.3)				114 ± 6.0 (5.3)
PCB-1260	99 ± 4.6 (4.6)				99 ± 4.6 (4.6)

[a] The values given represent the average percent recoveries from three replicate determinations ± one standard deviation; the numbers in parentheses are the relative standard deviations.

i -- Unable to determine the recovery because of interference.

TABLE 43. ELUTION PATTERNS AND AVERAGE RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs AFTER SILICA GEL CHROMATOGRAPHY (LIQUID WASTE NO. 1 EXTRACT) (CONTINUED)

Compound	Average ± SD (RSD) at Concentration 2[a]				
	Fraction I hexane (80 mL)	Fraction II hexane (50 mL)	Fraction III methylene chloride (15 mL)	Fraction IV ethyl acetate (50 mL)	Total recovery
alpha-BHC		60 ± 5.7 (9.5)	37 ± 7.4 (20)		97 ± 3.5 (3.6)
beta-BHC			100 ± 4.0 (4.0)		100 ± 4.0 (4.0)
gamma-BHC			100 ± 5.5 (5.5)		100 ± 5.5 (5.5)
delta-BHC			i		i
Heptachlor	94 ± 10 (11)				94 ± 1 (11)
Aldrin	98 ± 9.4 (9.6)				98 ± 9.4 (9.6)
Heptachlor epoxide			109 ± 14 (13)		109 ± 14 (13)
gamma-Chlordane	89 ± 6.5 (7.3)	13 ± 4.2 (32)			103 ± 2.5 (2.4)
Endosulfan I			100 ± 12 (12)		100 ± 12 (12)
4,4'-DDE	113 ± 2.5 (2.2)				113 ± 2.5 (2.2)
Dieldrin			86 ± 9.5 (11)		86 ± 9.5 (11)
Endrin			90 ± 9.9 (11)		90 ± 9.9 (11)
Endosulfan II			i		i
4,4'-DDD		37 ± 19 (50)	i		i
Endrin aldehyde			i		i
Kepone				0	0
Endosulfan sulfate			127 ± 22 (17)		127 ± 22 (17)
4,4'-DDT	83 ± 11 (13)				83 ± 11 (13)
4,4'-Methoxychlor			58 ± 9.3 (16)		58 ± 9.3 (16)
PCB-1016	122 ± 10 (8.3)				122 ± 10 (8.3)
PCB-1260	102 ± 4.7 (4.6)				102 ± 4.7 (4.6)

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[a] The values given represent the average percent recoveries from three replicate determinations ± one standard deviation; the numbers in parentheses are the relative standard deviations.

i -- Unable to determine the recovery because of interference.

TABLE 43. ELUTION PATTERNS AND AVERAGE RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs AFTER SILICA GEL CHROMATOGRAPHY (STANDARDS ONLY) (CONCLUDED)

	Average ± SD (RSD) at Concentration 3[a]				
Compound	Fraction I hexane (80 mL)	Fraction II hexane (50 mL)	Fraction III methylene chloride (15 mL)	Fraction IV ethyl acetate (50 mL)	Total recovery
alpha-BHC		57 ± 2.5 (4.4)	22 ± 9.2 (42)		79 ± 10 (13)
beta-BHC			90 ± 3.1 (3.4)		90 ± 3.1 (3.4)
gamma-BHC			90 ± 4.0 (4.4)		90 ± 4.0 (4.4)
delta-BHC			90 ± 11 (8.8)		90 ± 11 (8.8)
Heptachlor	90 ± 11 (12)				90 ± 11 (12)
Aldrin	92 ± 9.2 (10)				92 ± 9.2 (10)
Heptachlor epoxide			89 ± 4.1 (4.6)		89 ± 4.1 (4.6)
gamma-Chlordane	85 ± 7.2 (8.5)	10 ± 9.2 (92)			95 ± 8.0 (8.4)
Endosulfan I			88 ± 3.8 (4.3)		88 ± 3.8 (4.3)
4,4'-DDE	95 ± 16 (17)				95 ± 16 (17)
Dieldrin			82 ± 4.3 (5.3)		82 ± 4.3 (5.3)
Endrin			65 ± 3.1 (4.7)		65 ± 3.1 (4.7)
Endosulfan II			79 ± 7.1 (9.0)		79 ± 7.1 (9.0)
4,4'-DDD		33 ± 4.0 (15)	43 ± 16 (37)		76 ± 16 (21)
Endrin aldehyde			i		i[b]
Kepone				113; 223[b]	113; 223[b]
Endosulfan sulfate			83 ± 4.0 (4.8)		83 ± 4.0 (4.8)
4,4'-DDT	88 ± 18 (21)				88 ± 18 (21)
4,4'-Methoxychlor			75 ± 4.6 (6.1)		75 ± 4.6 (6.1)
PCB-1016	118 ± 9.8 (8.3)				118 ± 9.8 (8.3)
PCB-1260	100 ± 18 (18)				100 ± 18 (18)

[a] The values given represent the average percent recoveries from three replicate determinations ± one standard deviation; the numbers in parentheses are the relative standard deviations.

[b] Duplicate determinations.

i -- Unable to determine the recovery because of interference.

TABLE 44. ELUTION PATTERNS AND AVERAGE RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs AFTER SILICA GEL CHROMATOGRAPHY (NBS SEDIMENT EXTRACT)

	Average ± SD (RSD) at Concentration 1[a]				
Compound	Fraction I hexane (80 mL)	Fraction II hexane (50 mL)	Fraction III methylene chloride (15 mL)	Fraction IV ethyl acetate (50 mL)	Total recovery
alpha-BHC		52 ± 6.2 (12)	21 ± 6.1 (29)		73 ± 2.1 (2.9)
beta-BHC			88 ± 4.1 (4.7)		88 ± 4.1 (4.7)
gamma-BHC			83 ± 3.0 (3.6)		83 ± 3.0 (3.6)
delta-BHC			85 ± 4.6 (5.4)		85 ± 4.6 (5.4)
Heptachlor	53 ± 10 (19)				53 ± 10 (19)
Aldrin	69 ± 3.6 (5.2)				69 ± 3.6 (5.2)
Heptachlor epoxide			91 ± 4.9 (5.4)		91 ± 4.9 (5.4)
gamma-Chlordane	67 ± 7.4 (11)	10 ± 2.6 (26)			77 ± 5.3 (6.9)
Endosulfan I			85 ± 5.5 (6.5)		85 ± 5.5 (6.5)
4,4'-DDE	71 ± 7.1 (10)				71 ± 7.1 (10)
Dieldrin			92 ± 8.6 (9.4)		92 ± 8.6 (9.4)
Endrin			100 ± 9.5 (9.5)		100 ± 9.5 (9.5)
Endosulfan II			80 ± 7.4 (9.2)		80 ± 7.4 (9.2)
4,4'-DDD		37 ± 8.9 (37)	69 ± 12 (18)		106 ± 6.4 (6.0)
Endrin aldehyde			70 ± 5.7 (8.2)		70 ± 5.7 (8.2)
Kepone				0	0
Endosulfan sulfate			75 ± 9.0 (12)		75 ± 9.0 (12)
4,4'-DDT	54 ± 13 (24)				54 ± 13 (24)
4,4'-Methoxychlor					i
PCB-1016	104 ± 9.1 (8.7)				104 ± 9.1 (8.7)
PCB-1260	92 ± 9.2 (10)				92 ± 9.2 (10)

[a] The values given represent the average percent recoveries from three replicate determinations ± one standard deviation; the numbers in parentheses are the relative standard deviations.

i -- Unable to determine the recovery because of interference.

TABLE 44. ELUTION PATTERNS AND AVERAGE RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs AFTER SILICA GEL CHROMATOGRAPHY (NBS SEDIMENT EXTRACT) (CONTINUED)

Compound	Average ± SD (RSD) at Concentration 2[a] Fraction I hexane (80 mL)	Fraction II hexane (50 mL)	Fraction III methylene chloride (15 mL)	Fraction IV ethyl acetate (50 mL)	Total recovery
alpha-BHC		55 ± 6.1 (11)	20 ± 1.7 (8.7)		75 ± 6.0 (8.0)
beta-BHC			94 ± 3.0 (3.2)		94 ± 3.0 (3.2)
gamma-BHC			89 ± 4.1 (4.6)		89 ± 4.1 (4.6)
delta-BHC			92 ± 5.2 (5.6)		92 ± 5.2 (5.6)
Heptachlor	70 ± 7.7 (11)				70 ± 7.7 (11)
Aldrin	65 ± 4.6 (7.1)				65 ± 4.6 (7.1)
Heptachlor epoxide			91 ± 5.7 (6.3)		91 ± 5.7 (6.3)
gamma-Chlordane	71 ± 3.2 (4.5)	10 ± 2.0 (20)			81 ± 4.9 (6.1)
Endosulfan I			88 ± 5.1 (5.8)		88 ± 5.1 (5.8)
4,4'-DDE	76 ± 7.1 (9.3)				76 ± 7.1 (9.3)
Dieldrin			85 ± 9.4 (11)		85 ± 9.4 (11)
Endrin			87 ± 6.4 (7.3)		87 ± 6.4 (7.3)
Endosulfan II			81 ± 4.5 (5.5)		81 ± 4.5 (5.5)
4,4'-DDD		36 ± 2.0 (5.6)	49 ± 1.2 (2.4)		85 ± 3.1 (3.6)
Endrin aldehyde			71 ± 9.2 (13)		71 ± 9.2 (13)
Kepone				0	0
Endosulfan sulfate			86 ± 5.0 (5.8)		86 ± 5.0 (5.8)
4,4'-DDT	61 ± 7.9 (13)				61 ± 7.9 (13)
4,4'-Methoxychlor			99 ± 17 (17)		99 ± 17 (17)
PCB-1016	104 ± 2.5 (2.4)				104 ± 2.5 (2.4)
PCB-1260	95 ± 7.5 (7.9)				95 ± 7.5 (7.9)

[a] The values given represent the average percent recoveries from three replicate determinations ± one standard deviation; the numbers in parentheses are the relative standard deviations.

TABLE 44. ELUTION PATTERNS AND AVERAGE RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs AFTER SILICA GEL CHROMATOGRAPHY (NBS SEDIMENT EXTRACT) (CONCLUDED)

Compound	Fraction I hexane (80 mL)	Fraction II hexane (50 mL)	Fraction III methylene chloride (15 mL)	Fraction IV ethyl acetate (50 mL)	Total recovery
	Average ± SD (RSD) at Concentration 3[a]				
alpha-BHC		55 ± 3.6 (6.6)	21 ± 2.7 (13)		76 ± 5.6 (7.3)
beta-BHC			92 ± 7.1 (7.7)		92 ± 7.1 (7.7)
gamma-BHC			93 ± 8.1 (8.7)		93 ± 8.1 (8.7)
delta-BHC			94 ± 8.7 (9.3)		94 ± 8.7 (9.3)
Heptachlor	88 ± 4.1 (4.7)				88 ± 4.1 (4.7)
Aldrin	72 ± 1.0 (1.4)				72 ± 1.0 (1.4)
Heptachlor epoxide			93 ± 8.6 (9.2)		93 ± 8.6 (9.2)
gamma-Chlordane	75 ± 1.5 (2.0)	10 ± 2.9 (29)			85 ± 1.0 (1.2)
Endosulfan I			91 ± 9.1 (10)		91 ± 9.1 (10)
4,4'-DDE	84 ± 1.0 (1.2)				84 ± 1.0 (1.2)
Dieldrin			94 ± 10 (11)		94 ± 10 (11)
Endrin			76 ± 9.9 (13)		76 ± 9.9 (13)
Endosulfan II			91 ± 12 (13)		91 ± 12 (13)
4,4'-DDD		42 ± 2.3 (5.5)	47 ± 5.6 (12)		90 ± 7.2 (8.0)
Endrin aldehyde			88 ± 12 (14)		88 ± 12 (14)
Kepone				225 ± 99 (44)	225 ± 99 (44)
Endosulfan sulfate			72 ± 11 (15)		72 ± 11 (15)
4,4'-DDT	76 ± 2.5 (3.3)				76 ± 2.5 (3.3)
4,4'-Methoxychlor			92 ± 17 (19)		92 ± 17 (19)
PCB-1016	102 ± 4.6 (4.5)				102 ± 4.6 (4.5)
PCB-1260	91 ± 4.0 (4.4)				91 ± 4.0 (4.4)

[a] The values given represent the average percent recoveries from three replicate determinations ± one standard deviation; the numbers in parentheses are the relative standard deviations.

TABLE 45. ELUTION PATTERNS AND AVERAGE RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs AFTER SILICA GEL CHROMATOGRAPHY (SANDY LOAM EXTRACT)

Compound	Average ± SD (RSD) at Concentration 1[a]				
	Fraction I hexane (80 mL)	Fraction II hexane (50 mL)	Fraction III methylene chloride (15 mL)	Fraction IV ethyl acetate (50 mL)	Total recovery
alpha-BHC		61 ± 13 (21)	25 ± 3.5 (14)		86 ± 9.5 (11)
beta-BHC			94 ± 8.4 (8.9)		94 ± 8.4 (8.9)
gamma-BHC			92 ± 11 (12)		92 ± 11 (12)
delta-BHC			94 ± 12 (13)		94 ± 12 (13)
Heptachlor	89 ± 9.8 (11)				89 ± 9.8 (11)
Aldrin	99 ± 4.4 (4.4)				99 ± 4.4 (4.4)
Heptachlor epoxide			96 ± 11 (11)		96 ± 11 (11)
gamma-Chlordane	87 ± 9.6 (11)	13 ± 3.5 (27)			100 ± 8.3 (8.3)
Endosulfan I			95 ± 10 (11)		95 ± 10 (11)
4,4'-DDE	105 ± 14 (13)				105 ± 14 (13)
Dieldrin			113 ± 12 (11)		113 ± 12 (11)
Endrin			74 ± 5.6 (7.5)		74 ± 5.6 (7.5)
Endosulfan II			97 ± 14 (14)		97 ± 14 (14)
4,4'-DDD		42 ± 5.5 (13)	61 ± 4.1 (6.7)		103 ± 9.6 (9.3)
Endrin aldehyde			86 ± 11 (13)		86 ± 11 (13)
Kepone				0	0
Endosulfan sulfate			112 ± 19 (17)		112 ± 19 (17)
4,4'-DDT	107 ± 25 (23)				107 ± 25 (23)
4,4'-Methoxychlor			91 ± 14 (15)		91 ± 14 (15)
PCB-1016	90 ± 15 (17)				90 ± 15 (17)
PCB-1260	99 ± 6.8 (6.9)				99 ± 6.8 (6.9)

[a] The values given represent the average percent recoveries from three replicate determinations ± one standard deviation; the numbers in parentheses are the relative standard deviations.

TABLE 45. ELUTION PATTERNS AND AVERAGE RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs AFTER SILICA GEL CHROMATOGRAPHY (SANDY LOAM EXTRACT) (CONTINUED)

	Average ± SD (RSD) at Concentration 2[a]				
Compound	Fraction I hexane (80 mL)	Fraction II hexane (50 mL)	Fraction III methylene chloride (15 mL)	Fraction IV ethyl acetate (50 mL)	Total recovery
alpha-BHC		49 ± 3.5 (7.1)	38 ± 4.2 (11)		87 ± 4.9 (5.7)
beta-BHC			90 ± 4.1 (4.5)		90 ± 4.1 (4.5)
gamma-BHC			91 ± 3.0 (3.3)		91 ± 3.0 (3.3)
delta-BHC			89 ± 3.6 (4.1)		89 ± 3.6 (4.1)
Heptachlor	83 ± 9.1 (11)				83 ± 9.1 (11)
Aldrin	88 ± 2.0 (2.3)				88 ± 2.0 (2.3)
Heptachlor epoxide			90 ± 4.6 (5.1)		90 ± 4.6 (5.1)
gamma-Chlordane	70 ± 6.0 (8.6)	23 ± 5.1 (22)			93 ± 3.0 (3.2)
Endosulfan I			89 ± 5.3 (5.9)		89 ± 5.3 (5.9)
4,4'-DDE	93 ± 5.6 (6.0)				93 ± 5.6 (6.0)
Dieldrin			99 ± 4.6 (4.6)		99 ± 4.6 (4.6)
Endrin			60 ± 7.8 (13)		60 ± 7.8 (13)
Endosulfan II			86 ± 6.7 (7.8)		86 ± 6.7 (7.8)
4,4'-DDD		21 ± 1.2 (5.6)	67 ± 7.4 (11)		88 ± 7.8 (8.9)
Endrin aldehyde			82 ± 7.6 (9.3)		82 ± 7.6 (9.3)
Kepone				0	0
Endosulfan sulfate			91 ± 13 (14)		91 ± 13 (14)
4,4'-DDT	83 ± 5.3 (6.4)				83 ± 5.3 (6.4)
4,4'-Methoxychlor			89 ± 9.8 (11)		89 ± 9.8 (11)
PCB-1016	92 ± 4.5 (4.9)				92 ± 4.5 (4.9)
PCB-1260	89 ± 8.9 (10)				89 ± 8.9 (10)

[a] The values given represent the average percent recoveries from three replicate determinations ± one standard deviation; the numbers in parentheses are the relative standard deviations.

TABLE 45. ELUTION PATTERNS AND AVERAGE RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs AFTER SILICA GEL CHROMATOGRAPHY (SANDY LOAM EXTRACT) (CONCLUDED)

Compound	Average ± SD (RSD) at Concentration 3[a]				
	Fraction I hexane (80 mL)	Fraction II hexane (50 mL)	Fraction III methylene chloride (15 mL)	Fraction IV ethyl acetate (50 mL)	Total recovery
alpha-BHC		46 ± 4.6 (10)	43 ± 2.0 (4.8)		89 ± 2.5 (2.8)
beta-BHC			93 ± 5.0 (5.4)		93 ± 5.0 (5.4)
gamma-BHC			95 ± 4.6 (4.8)		95 ± 4.6 (4.8)
delta-BHC			93 ± 6.1 (6.5)		93 ± 6.1 (6.5)
Heptachlor	79 ± 17 (21)				79 ± 17 (21)
Aldrin	82 ± 12 (15)				82 ± 12 (15)
Heptachlor epoxide			94 ± 6.6 (7.0)		94 ± 6.6 (7.0)
gamma-Chlordane	63 ± 8.8 (14)	24 ± 2.4 (10)			87 ± 11 (13)
Endosulfan I			93 ± 7.5 (8.1)		93 ± 7.5 (8.1)
4,4'-DDE	82 ± 11 (13)				82 ± 11 (13)
Dieldrin			93 ± 8.2 (8.8)		93 ± 8.2 (8.8)
Endrin			47 ± 11 (24)		47 ± 11 (24)
Endosulfan II			89 ± 11 (12)		89 ± 11 (12)
4,4'-DDD		18 ± 2.7 (15)	72 ± 5.2 (7.2)		90 ± 7.8 (8.6)
Endrin aldehyde			73 ± 20 (28)		73 ± 20 (28)
Kepone				260 ± 55 (21)	260 ± 55 (21)
Endosulfan sulfate			88 ± 11 (13)		88 ± 11 (13)
4,4'-DDT	64 ± 13 (21)				64 ± 12 (21)
4,4'-Methoxychlor			86 ± 14 (16)		86 ± 14 (16)
PCB-1016	85 ± 10 (12)				85 ± 10 (12)
PCB-1260	85 ± 13 (15)				85 ± 13 (15)

[a] The values given represent the average percent recoveries from three replicate determinations ± one standard deviation; the numbers in parentheses are the relative standard deviations.

TABLE 46. ELUTION PATTERNS AND AVERAGE RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs AFTER SILICA GEL CHROMATOGRAPHY (SOIL EXTRACT CONTAMINATED WITH TOXAPHENE)

Compound	Average ± SD (RSD) at Concentration 1[a]				
	Fraction I hexane (80 mL)	Fraction II hexane (50 mL)	Fraction III methylene chloride (15 mL)	Fraction IV ethyl acetate (50 mL)	Total recovery
alpha-BHC		67 ± 15 (22)	12 ± 3.7 (31)		79 ± 18 (22)
beta-BHC			97 ± 6.5 (6.7)		97 ± 6.5 (6.7)
gamma-BHC			86 ± 12 (14)		86 ± 12 (14)
delta-BHC			95 ± 11 (12)		95 ± 11 (12)
Heptachlor	82 ± 9.0 (11)				82 ± 9.0 (11)
Aldrin	84 ± 7.6 (9.1)				84 ± 7.6 (9.1)
Heptachlor epoxide			103 ± 15 (15)		103 ± 15 (15)
gamma-Chlordane	83 ± 7.1 (8.5)	10 ± 2.0 (20)			93 ± 8.5 (9.1)
Endosulfan I			100 ± 14 (14)		100 ± 14 (14)
4,4'-DDE	70 ± 0.6 (0.8)				70 ± 0.6 (0.8)
Dieldrin			103 ± 16 (15)		103 ± 16 (15)
Endrin			93 ± 10 (11)		93 ± 10 (11)
Endosulfan II			100 ± 13 (13)		100 ± 13 (13)
4,4'-DDD		66 ± 11 (17)	30 ± 6.6 (22)		96 ± 18 (18)
Endrin aldehyde			91 ± 14 (15)		91 ± 14 (15)
Kepone				NA	NA
Endosulfan sulfate			127 ± 26 (20)		127 ± 26 (20)
4,4'-DDT	66 ± 11 (17)				66 ± 11 (17)
4,4'-Methoxychlor			99 ± 12 (12)		99 ± 12 (12)
PCB-1016	89 ± 20 (22)				89 ± 20 (22)
PCB-1260	89 ± 23 (26)				89 ± 23 (26)

NA -- not analyzed.

[a] The values given represent the average percent recoveries from three replicate determinations ± one standard deviation; the numbers in parentheses are the relative standard deviations.

TABLE 46. ELUTION PATTERNS AND AVERAGE RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs AFTER SILICA GEL CHROMATOGRAPHY (SOIL EXTRACT CONTAMINATED WITH TOXAPHENE) (CONTINUED)

Compound	Average ± SD (RSD) at Concentration 2[a]				
	Fraction I hexane (80 mL)	Fraction II hexane (50 mL)	Fraction III methylene chloride[b] (15 mL)	Fraction IV ethyl acetate (50 mL)	Total recovery
alpha-BHC		48 ± 4.7 (10)	4.0 ; 6.7		47 ; 58
beta-BHC			73 ; 74		73 ; 74
gamma-BHC			53 ; 63		53 ; 63
delta-BHC			68 ; 74		68 ; 74
Heptachlor	74 ± 12 (16)				74 ± 12 (16)
Aldrin	82 ± 5.6 (6.8)				82 ± 5.6 (6.8)
Heptachlor epoxide			74 ; 74		74 ; 74
gamma-Chlordane	83 ± 6.8 (8.2)	4.9 ± 0.7 (14)			87 ± 6.4 (7.4)
Endosulfan I			68 ; 70		68 ; 70
4,4'-DDE	71 ± 12 (17)				71 ± 12 (17)
Dieldrin			71 ; 72		71 ; 72
Endrin			63 ; 67		63 ; 67
Endosulfan II			70 ; 70		70 ; 70
4,4'-DDD		50 ± 5.9 (12)	11 ; 12		57 ; 69
Endrin aldehyde			59 ; 68		59 ; 68
Kepone				NA	NA
Endosulfan sulfate			88 ; 73		88 ; 73
4,4'-DDT	56 ± 23 (41)				56 ± 23 (41)
4,4'-Methoxychlor			70 ; 70		70 ; 70
PCB-1016	98 ± 23 (23)				98 ± 23 (23)
PCB-1260	77 ± 18 (23)				77 ± 18 (23)

NA -- not analyzed.

[a] The values given represent the average percent recoveries from three replicate determinations ± one standard deviation; the numbers in parentheses are the relative standard deviations.

[b] The values given represent duplicate determinations.

TABLE 46. ELUTION PATTERNS AND AVERAGE RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs AFTER SILICA GEL CHROMATOGRAPHY (SOIL EXTRACT CONTAMINATED WITH TOXAPHENE) (CONCLUDED)

Compound	Average ± SD (RSD) at Concentration 3[a]				
	Fraction I hexane (80 mL)	Fraction II hexane (50 mL)	Fraction III methylene chloride (15 mL)	Fraction IV ethyl acetate (50 mL)	Total recovery
alpha-BHC		61 ± 5.5 (9.0)	11 ± 2.3 (21)		73 ± 5.9 (8.0)
beta-BHC			87 ± 6.5 (7.5)		87 ± 6.5 (7.5)
gamma-BHC			78 ± 12 (15)		78 ± 12 (15)
delta-BHC			89 ± 7.5 (8.4)		89 ± 7.5 (8.4)
Heptachlor	63 ± 8.7 (14)				63 ± 8.7 (14)
Aldrin	83 ± 6.2 (7.5)				83 ± 6.2 (7.5)
Heptachlor epoxide			93 ± 2.6 (2.8)		93 ± 2.6 (2.8)
gamma-Chlordane	80 ± 7.6 (9.5)	7.0 ± 1.1 (16)			87 ± 6.8 (7.8)
Endosulfan I			84 ± 7.2 (8.6)		84 ± 7.2 (8.6)
4,4'-DDE	81 ± 9.7 (12)				81 ± 9.7 (12)
Dieldrin			88 ± 8.5 (9.7)		88 ± 8.5 (9.7)
Endrin			77 ± 7.0 (9.1)		77 ± 7.0 (9.1)
Endosulfan II			85 ± 8.1 (9.6)		85 ± 8.1 (9.6)
4,4'-DDD		58 ± 4.5 (7.8)	24 ± 4.0 (17)		82 ± 7.2 (8.8)
Endrin aldehyde			84 ± 8.7 (10)		84 ± 8.7 (10)
Kepone				NA	NA ±
Endosulfan sulfate			87 ± 9.1 (10)		87 ± 9.1 (10)
4,4'-DDT	72 ± 8.9 (12)				72 ± 8.9 (12)
4,4'-Methoxychlor			85 ± 11 (12)		85 ± 11 (12)
PCB-1016	53 ± 3.5 (6.6)				53 ± 3.5 (6.6)
PCB-1260	49 ± 5.3 (11)				49 ± 5.3 (11)

NA -- not analyzed.

[a] The values given represent the average percent recoveries from three replicate determinations ± one standard deviation; the numbers in parentheses are the relative standard deviations.

[b] The values given represent duplicate determinations.

analysis allows complete separation of the five organochlorine pesticides that elute with the PCBs in Fraction 1.

7.3 METHOD DETECTION LIMIT

The determination of the method detection limit was performed according to the following procedure:

- Make an estimate of the detection limit from the concentration value that corresponds to an instrument signal-to-noise ratio in the range of 5 to 10.
- Prepare reagent water or soil at a concentration that is in the same concentration range as the estimated method detection limit; in this case the concentration is 0.1 µg/L for water (except PCB-1016 and PCB-1260 at 1.0 µg/L) and 10 µg/Kg for soil (except PCB-1016 and PCB-1260 at 100 µg/Kg).
- Take seven aliquots of the matrix and process each aliquot through the entire analytical method. Make all computations according to the protocol written for the revised Method 8080. A blank measurement is also performed to calculate the concentration of the compound in the unspiked sample and to subtract it from the respective spiked sample measurements.
- Calculate the standard deviation (SD) of the seven replicate measurements and compute the MDL as follows:

$$\text{MDL} = t_{(n-1,\ 0.99)} \times \text{SD}$$

where $t_{(n-1,\ 0.99)}$ is the student's t value appropriate for a 99% confidence level and a standard deviation with n-1 degrees of freedom, and SD is the standard deviation of the seven replicate measurements. The value of t for 6 degrees of freedom is 3.143; a t value of 3 was used to compute the MDL values in Tables 47 and 48 for water and soil samples, respectively.

Data are not available for either Kepone or toxaphene. Kepone was not detected in any of the seven replicates, and toxaphene was not spiked into any of the samples since it would interfere with the determination of the organochlorine pesticides and PCBs.

TABLE 47. RESULTS OF METHOD DETECTION LIMIT (MDL) DETERMINATION FOR ORGANOCHLORINE PESTICIDES AND PCBs IN DISTILLED WATER

Compound	Amount spiked (µg/L)	Amount found in a given spiked replicate (µg/L) (1)	(2)	(3)	(4)	(5)	(6)	(7)	Average	SD	MDL	Average percent recovery
alpha-BHC	0.100	0.057	0.086	0.069	0.085	0.059	0.062	0.069	0.070	0.012	0.035	70
beta-BHC	0.100	0.077	0.098	0.083	0.093	0.095	0.093	0.083	0.089	0.008	0.023	89
gamma-BHC	0.100	0.083	0.102	0.089	0.102	0.091	0.090	0.081	0.091	0.008	0.025	91
delta-BHC	0.100	0.076	0.099	0.085	0.095	0.096	0.091	0.085	0.090	0.008	0.024	90
Heptachlor	0.100	0.060	0.083	0.070	0.096	0.095	0.089	0.076	0.081	0.013	0.040	81
Aldrin	0.100	0.052	0.058	0.046	0.073	0.074	0.074	0.062	0.063	0.011	0.034	63
Heptachlor epoxide	0.100	0.070	0.088	0.080	0.084	0.102	0.095	0.094	0.088	0.011	0.032	88
gamma-Chlordane	0.100	0.050	0.051	0.046	0.063	0.076	0.071	0.046	0.058	0.012	0.037	58
Endosulfan I	0.100	0.075	0.092	0.080	0.086	0.103	0.095	0.098	0.090	0.010	0.030	90
4,4'-DDE	0.100	0.066	0.075	0.062	0.100	0.108	0.104	0.073	0.084	0.019	0.058	84
Dieldrin	0.100	0.077	0.094	0.085	0.084	0.116	0.104	0.111	0.096	0.015	0.044	96
Endrin	0.100	0.083	0.108	0.094	0.078	0.096	0.069	0.085	0.088	0.013	0.039	88
Endosulfan II	0.100	0.075	0.092	0.081	0.082	0.108	0.094	0.110	0.092	0.013	0.040	92
4,4'-DDD	0.100	0.074	0.099	0.090	0.089	0.100	0.093	0.129	0.096	0.017	0.050	96
Endrin aldehyde	0.100	0.054	0.072	0.066	0.066	0.092	0.079	0.103	0.076	0.017	0.050	76
Endosulfan sulfate	0.100	0.080	0.103	0.092	0.085	0.102	0.089	0.113	0.095	0.012	0.035	95
4,4'-DDT	0.100	0.057	0.077	0.062	0.112	0.120	0.106	0.061	0.085	0.027	0.081	85
4,4'-Methoxychlor	0.100	0.125	0.180	0.155	0.134	0.115	0.090	0.137	0.134	0.029	0.086	134
PCB-1016	1.0	0.74	0.81	0.68	1.2	1.0	0.80	1.0	0.89	0.18	0.54	89
PCB-1260	1.0	0.40	0.96	0.84	1.3	1.1	1.1	0.73	0.92	0.30	0.90	92

TABLE 48. RESULTS OF THE METHOD DETECTION LIMIT (MDL) DETERMINATION FOR ORGANOCHLORINE PESTICIDES AND PCBs IN SANDY LOAM SOIL

Compound	Amount spiked (μg/Kg)	Amount found in a given spiked replicate (μg/kg) (1)	(2)	(3)	(4)	(5)	(6)	(7)	Average	SD	MDL	Average percent recovery
alpha-BHC	10	1.5	0.9	1.6	0.8	1.6	1.1	2.7	1.5	0.64	1.9	15
beta-BHC	10	3.9	3.1	3.5	4.8	6.3	3.6	4.3	4.2	1.1	3.3	42
gamma-BHC	10	4.1	2.6	3.8	3.8	4.8	3.5	3.7	3.8	0.66	2.0	38
delta-BHC	10	4.3	4.4	3.7	4.1	4.2	3.8	4.8	4.2	0.37	1.1	42
Heptachlor	10	4.8	4.5	3.9	3.4	2.9	3.4	3.5	3.8	0.67	2.0	38
Aldrin	10	3.7	3.4	2.7	2.2	1.8	2.1	2.1	2.6	0.73	2.2	26
Heptachlor epoxide	10	4.6	3.6	3.8	3.8	3.8	3.7	5.5	4.1	0.69	2.1	41
gamma-Chlordane	10	3.4	3.2	3.2	2.8	2.6	4.2	3.3	3.2	0.51	1.5	32
Endosulfan I	10	4.5	4.4	3.7	3.5	3.7	3.7	5.5	4.1	0.71	2.1	41
4,4'-DDE	10	4.4	4.6	3.8	4.1	3.9	6.0	5.5	4.6	0.84	2.5	46
Dieldrin	10	NA	NA	NA	NA	NA	NA	NA	NA	--	--	--
Endrin	10	3.6	3.5	3.0	2.7	3.8	3.6	6.5	3.8	1.2	3.6	38
Endosulfan II	10	4.7	4.9	3.8	3.8	4.2	4.0	6.0	4.5	0.79	2.4	45
4,4'-DDD	10	4.0	4.4	3.1	3.0	3.8	2.8	6.9	4.0	1.4	4.2	40
Endrin aldehyde	10	2.7	3.1	2.6	2.6	2.6	2.9	4.1	2.9	0.54	1.6	29
Endosulfan sulfate	10	4.5	5.5	3.4	4.1	4.4	3.7	7.0	4.7	1.2	3.6	47
4,4'-DDT	10	4.0	4.5	3.9	3.6	3.7	6.5	6.0	4.6	1.2	3.6	46
4,4'-Methoxychlor	10	4.3	4.3	3.1	3.9	5.0	4.0	9.0	4.8	1.9	5.7	48
PCB-1016	100	150	130	120	110	89	120	110	120	19	57	120
PCB-1260	100	130	100	88	85	73	130	120	100	23	70	100

NA -- Not able to determine because of matrix interference.

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APPENDIX A

LITERATURE REVIEW

TABLE OF CONTENTS

Section Page

1 Structures 110

2 Analytical Methodologies for Organochlorine Pesticides and PCBs 116

2.1 Sample Preservation. 116
2.2 Extraction 117

2.2.1 Extraction of Water Samples 120
2.2.2 Extraction of Sediment and Soil Samples 125

2.3 Extract Cleanup. 135

2.3.1 Liquid-Liquid Partitioning. 135
2.3.2 Gel Permeation Chromatography 137
2.3.3 Sulfur Removal. 137
2.3.4 Liquid-Solid Chromatography 140
2.3.5 High-Pressure Liquid Chromatography 149
2.3.6 Selection of the Cleanup Technique. 152

2.4 GC Analysis. 152

2.4.1 Gas Chromatographic Columns 156
2.4.2 Problems With Chromatography. 159

2.5 Confirmation of Compound Identity. 163
2.6 Stability of Pesticide Solutions 168

FIGURES

Number Page

A-1 Effect of silicic acid water content on elution and separation of PCB-1260 and DDT analogs. 147

A-2 Fractionation scheme employing Woelm neutral alumina, activity I, and silica gel deactivated with 3 percent water for the separation of organochlorine pesticides and PCBs. . . . 153

A-3 Fractionation of organochlorine pesticides, PCBs, PCNs, and phthalate esters using neutral alumina, activity I, and silica gel deactivated with 0.3 percent water 154

A-4 Florisil/charcoal column fractionation of organochlorine pesticides and PCBs 155

A-5 1.5 percent OV-17 + 1.95 percent OF-1 158

A-6 4 percent SE-30 + 6 percent OV-210. 158

A-7 5 percent OV-210. 158

A-8 3 percent DEGS. 160

A-9 10 percent DC-200 160

A-10 1.6 percent OF-17 + 6.4 percent OV-210. 160

A-11 5 percent DC-200 + 7.5 percent OF-1 160

A-12 GC/EC chromatogram of composite organochlorine pesticide and PCB-1016/1260 standard (30 m x 0.25 mm ID DB-5 fused-silica capillary column; 100 to 200 pg pesticides 1,000 PCBs injected). 161

A-13 GC/EC chromatogram of composite organochlorine pesticide and PCB-1016/1260 standard (1.8 m x 4 mm ID packed 1.5 percent OV-17 + 1.95 percent OV-210, 25 to 50 pg injected for pesticides and 500 pg injected for PCBs). 162

FIGURES (Continued)

Number Page

A-14 Chromatograms illustrating column overloading and subsequent rejuvenation 164

A-15 Reduction in decomposition of endrin resulting from column silylation 167

A-16 Comparative evaporation rates (mL/wk) of hexane and isooctane from different containers at ambient temperature. . . 175

TABLES

Number Page

A-1 Nomenclatures, Structures, Formulae, and Molecular Weights of the Organochlorine Pesticides. 111

A-2 Mean Recoveries from Preservation Tests in Clean Water after 7 days at 4°C 118

A-3 Mean Recoveries from Preservation Tests in Clean Water after 7 days at 24°C. 119

A-4 Extraction of Organochlorine Pesticides from Water Samples 121

A-5 Organochlorine Pesticides and PCBs Recoveries by Extraction with Methylene Chloride from Water 122

A-6 Recoveries from Wastewaters (Percent of Spiked Amount). 123

A-7 Recoveries of PCBs and Pesticides from Water. 124

A-8 Extraction of Organochlorine Pesticides from Soil and Sediment Samples. 126

A-9 Recoveries of Organochlorine Pesticides Added to Christiana Clay Loam. 127

A-10 Comparison of Extraction Efficiencies 129

A-11 Comparison of Extraction Methods for Efficiency of Extraction of Heptachlor Epoxide and Dieldrin from a Wet and Dry Clay Soil Using Acetone 130

A-12 Comparison of Extraction Efficiencies of Solvents 132

A-13 Extraction of Contaminated Bottom Material. 133

A-14 Influence of Moisture Content 134

A-15 Effect of Water on Recovery in ppm of Organochlorine Pesticides from Two Samples of Silt Loam Soil by Soxhlet Extraction. 136

TABLES (Continued)

Number		Page
A-16	Effect of Amount of Water Added to an Air-Dried Clay Loam Soil on the Recovery of Chlordane Residues by 1:1 Hexane:Acetone (Mechanical Shaker Method)	136
A-17	Solvent Partition of Pesticides and Butter Oil Between 1 Part Hexane and 3 Parts Polar Solvent	137
A-18	Elution Volumes of Organochlorine Pesticides by Gel Permeation Chromatography	138
A-19	Effect of Exposure of Organochlorine Pesticides to Various Sulfur Removal Reagents	139
A-20	Results of Recovery Experiments from Spiked Sediments Using Tetrabutyl Ammonium Sulfite	140
A-21	Column Chromatographic Cleanup of Organochlorine Pesticides and PCBs Using Florisil.	141
A-22	Mills 1972 Modified Procedure for Elution of Organochlorine Pesticides from Florisil	143
A-23	Pesticide Recoveries and Fat Elution from Florisil of Different Calcinations.	145
A-24	Elution Patterns of Organochlorine Pesticides and PCBs Using Armour/Burke's Procedure with Silica Gel/Celite 545 . . .	146
A-25	Elution Patterns of Organochlorine Pesticides and PCBs Using Leoni's Silica Gel Procedure (5 Percent Deactivated). . .	148
A-26	Elution Patterns of Organochlorine Pesticides and PCBs Using Biddleman's Silica Gel Procedure (Modified by Acurex) . .	149
A-27	Column Chromatographic Cleanup of Organochlorine Pesticides and PCBs Using Silica Gel.	150
A-28	Column Chromatographic Cleanup of Organochlorine Pesticides and PCBs Using Alumina	151

TABLES (Continued)

Number		Page
A-29	Relative Retention Times of Some Common Organochlorine Pesticides.	157
A-30	Summary of Pesticide Retention Times (min) on Two Fused-Silica Capillary Columns.	163
A-31	On-Column Decomposition of 4,4'-DDT	165
A-32	On-Column Decomposition of Endrin	166
A-33	Type of Reactions Used for the Confirmation of Organochlorine Pesticides by Chemical Derivatization -- GC Techniques	169
A-34	Applications of Alkali Dehydrochlorination for DDT and Analogs	170
A-35	Summary of Chemical Confirmation Procedures for Endrin and Dieldrin.	171
A-36	Summary of Chemical Confirmation Procedures for Aldrin.	171
A-37	Summary of Chemical Confirmation Procedures for the Chlordane Group	172
A-38	Chemical Confirmation of BHC Isomers.	173
A-39	Summary of Chemical Confirmation Procedures for Endosulfans	173
A-40	Evaporation Rates from 10-mL Glass-Stopper Volumetric Flasks and Vapor Pressure/Surface Tension Ratios for Some Common Organic Solvents.	174
A-41	Organochlorine Isooctane Solutions and 2-Year Stability Test Results.	177

SECTION 1

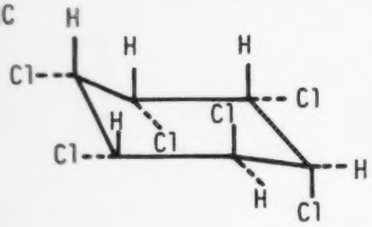
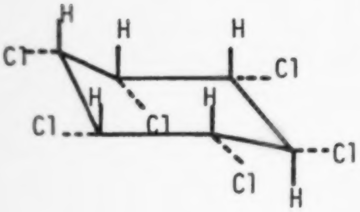
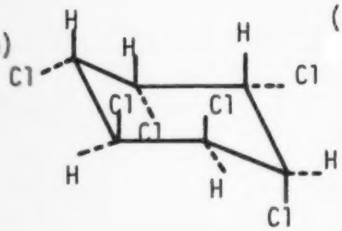
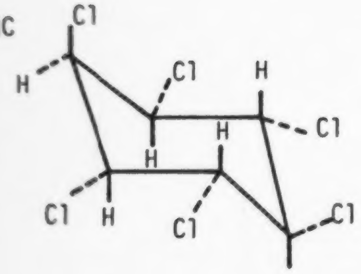
STRUCTURES

The chemical structures, common names, formulae, molecular weights, and nomenclatures of the organochlorine pesticides listed in Method 8080 are given in Table A-1.

Organochlorine pesticides consist of three different major groups based on their molecular structures: the cyclopentadiene group, the cyclohexane group, and the DDT group. Except for toxaphene, the cyclopentadiene pesticides are the Diels-Alder reaction products of hexachlorocyclopentadiene and suitable unsaturated compounds. Toxaphene is a mixture of chlorinated camphenes containing approximately 67 to 69 percent chlorine.

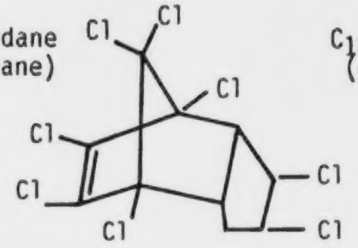
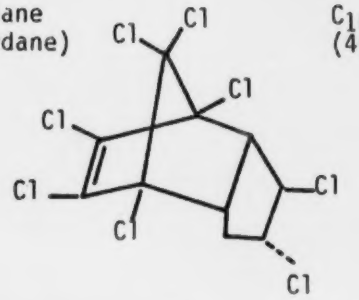
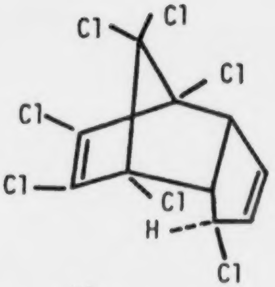
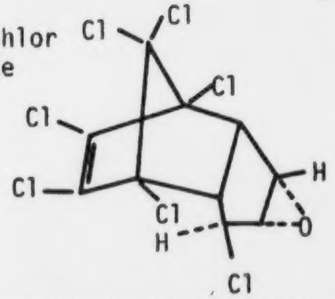
PCBs are complex mixtures of chlorinated biphenyls containing up to 209 isomers. Commercial mixtures of PCBs are known as Aroclors 1016, 1221, 1232, 1242, 1248, 1254, and 1260 (the last two digits indicate the percentage of chlorine in the mixture, except for Aroclor 1016 which contains 41.5 percent chlorine). In addition to PCBs, commercial preparations also contain chlorinated naphthalenes and traces of chlorinated dibenzofurans.

TABLE A-1. NOMENCLATURES, STRUCTURES, FORMULAE, AND MOLECULAR WEIGHTS OF THE ORGANOCHLORINE PESTICIDES

	Common name	Structure	Formula and mol. wt.	Nomenclature
1.	alpha-BHC		$C_6H_6Cl_6$ (290.8)	alpha-1,2,3,4,5,6-hexachlorocyclohexane
2.	beta-BHC		$C_6H_6Cl_6$ (290.8)	beta-1,2,3,4,5,6-hexachlorocyclohexane
3.	gamma-BHC (lindane) (active form)		$C_6H_6Cl_6$ (290.8)	gamma-1,2,3,4,5,6-hexachlorocyclohexane
4.	delta-BHC		$C_6H_6Cl_6$ (290.8)	delta-1,2,3,4,5,6-hexachlorocyclohexane

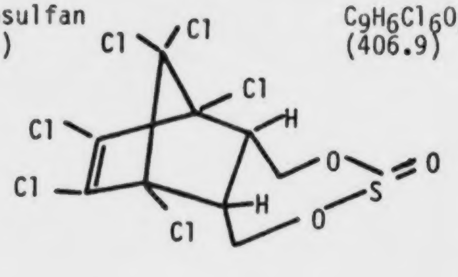
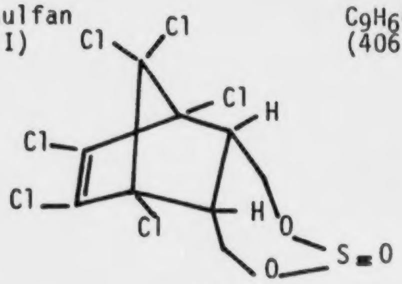
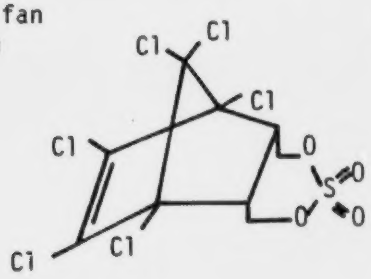
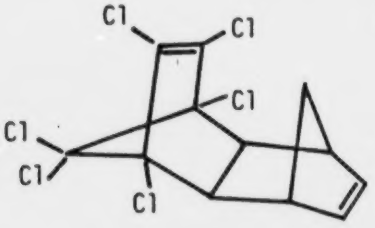
(continued)

TABLE A-1. (continued)

	Common name	Structure	Formula and mol. wt.	Nomenclature
5.	alpha-Chlordane (cis-Chlordane)		$C_{10}H_6Cl_8$ (409.8)	1-exo, 2-exo, 4,5,6,7,8,8-octachloro 2,3,3a,4,7,7a-hexahydro-4,7-methanoindene
6.	gamma-Chlordane (trans-Chlordane)		$C_{10}H_6Cl_8$ (409.8)	1-exo, 2-endo, 4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene
7.	Heptachlor		$C_{10}H_5Cl_7$ (373.3)	1,4,5,6,7,8,8-heptachlor-3a,4,7,7a-tetrahydro-4,7-methanoindene or 1,4,5,6,7,8,8-heptachloro-4,7-endomethylene-3a,4,7,7a-tetrahydroindene
8.	Heptachlor epoxide		$C_{10}H_5Cl_7O$ (389.3)	2,3-epoxy-1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene

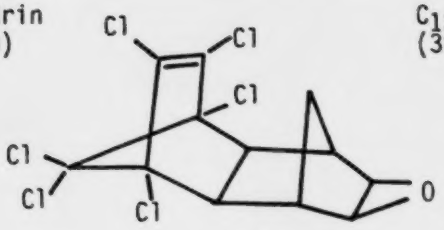
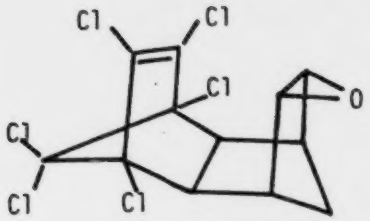
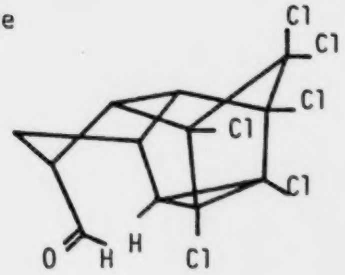
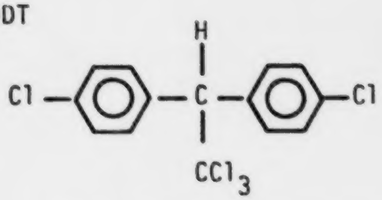
(continued)

TABLE A-1. (continued)

Common name	Structure	Formula and mol. wt.	Nomenclature
9. alpha-Endosulfan (Thiodan-I)		$C_9H_6Cl_6O_3S$ (406.9)	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide (α-isomer)
10. beta-Endosulfan (Thiodan-II)		$C_9H_6Cl_6O_3S$ (406.9)	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide (β-isomer)
11. Endosulfan sulfate		$C_9H_6Cl_6O_4S$ (422.9)	6,9-methano-2,4,3-benzodioxathiepin, 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-3,3-dioxide
12. Aldrin		$C_{12}H_8Cl_6$ (364.9)	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-endo-1, 4-exo-5,8-dimethanonaphthalene

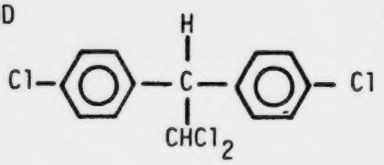
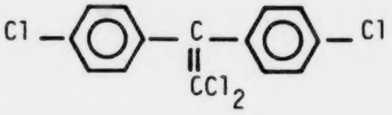
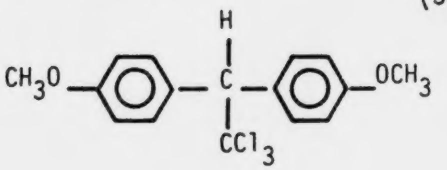
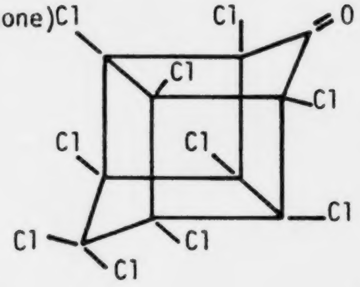
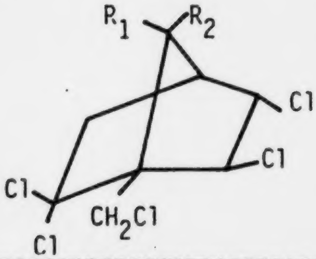
(continued)

TABLE A-1. (continued)

Common name	Structure	Formula and mol. wt.	Nomenclature
13. Dieldrin (HEOD)		$C_{12}H_8Cl_6O$ (380.9)	1,2,3,4,10,10-hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo, exo-5,8-dimethanonaphthalene
14. Endrin		$C_{12}H_8Cl_6O$ (380.9)	1,2,3,4,10,10-hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo, endo-5,8-dimethanonaphthalene
15. Endrin aldehyde		$C_{12}H_8Cl_6O$ (380.9)	1,2,4-methenocyclopenta (cd)pentalene-5-carboxaldehyde, 2,2a,3,4,7-hexachlorodecahydro-, (1α,2β,2aβ,4β,4aβ,5β,6aβ,6bβ,7R*)-
16. 4,4'-DDT		$C_{14}H_9Cl_5$ (354.5)	1,1,1-trichloro-2,2-bis (p-chlorophenyl)-ethane

(continued)

TABLE A-1. (concluded)

	Common name	Structure	Formula and mol. wt.	Nomenclature
17.	4,4'-DDD (TDE)		$C_{14}H_{10}Cl_4$ (320.1)	1,1-dichloro-2,2-bis (p-chlorophenyl)ethane
18.	4,4'-DDE		$C_{14}H_8Cl_4$ (318.1)	1,1-dichloro-2,2-bis (p-chlorophenyl)ethane
19.	4,4'-Methoxychlor		$C_{16}H_{15}Cl_3O_2$ (345.7)	1,1,1-trichloro-2,2-bis (p-methoxyphenyl)ethane
20.	Kepone (chlordecone)		$C_{10}Cl_{10}O$ (490.6)	1,2,3,5,6,7,8,9,10,10-decachloro {5.2.1.$0^{2.6}$ $0^{3.9}0^{5.8}$} decano-4-one or decachlorooctahydro 1,3,4-methano-2H-cyclobuta {cd}-pentalen-2-one
21.	Toxaphene		$C_{10}H_{10}Cl_8$ (413.8)	Toxaphene [mixture of chlorinated camphenes (chlorine 67-69 percent) of uncertain identity]

SECTION 2

ANALYTICAL METHODOLOGIES FOR ORGANOCHLORINE PESTICIDES AND PCBs

The discussion of analytical methodologies for organochlorine pesticides and PCBs addresses the following: sample preservation, extraction, cleanup, GC analysis, compound confirmation, and stability of organochlorine pesticide solutions.

2.1 SAMPLE PRESERVATION

The choice of the preservation method depends on the type of sample, the compounds to be determined, the duration of sample storage prior to analysis, and the analytical procedure to be used. The method chosen must not impair the analytical procedure to be used in determining the compound.

The containers in which the samples are collected must be made of suitable material for the preservation method and for the chemical to be determined. Narrow-mouth glass bottles with aluminum-foil-lined lids are recommended for the collection of water samples (1). Also, emptied 1-gal amber solvent bottles previously used for acetone, hexane, and petroleum ether can be used for sample collection. The use of Teflon liners instead of aluminum foil is not recommended because of possible adsorption of some pesticides onto Teflon (1).

Weil and Quentin (2) investigated the effect of container, temperature, and light on the storage of water samples containing DDT, dieldrin, heptachlor epoxide, lindane, and methoxychlor at 10 µg/L and reported substantial losses of the test compounds during a 2-week storage period in polyethylene containers. For aluminum and glass containers, the recoveries were in the range of 40 to 90 and 50 to 100 percent, respectively. Small losses of DDT, dieldrin, and heptachlor epoxide were reported from samples which were stored at room temperature and in sunlight.

In another study, dealing with the persistence of organochlorine pesticides in river water, Eichelberger and Lichtenberg (3) reported that heptachlor, aldrin, alpha- and beta-endosulfan, and chlordane degrade totally or partially during an 8-week storage period in glass containers in sunlight and fluorescent light.

The effects of pH, temperature, and residual chlorine on the preservation of spiked water samples in glass bottles sealed with aluminum-foil-lined caps for a period of 7 days were investigated by Millar et al. (4). The results of Millar's study are given in Tables A-2 and A-3 and are summarized briefly as follows:

- The best overall conditions for the storage of water samples containing organochlorine pesticides and PCBs are 4°C and pH 2.
- From water samples stored at 4°C, recoveries at pH 7 were similar to those at pH 2 and pH 10, except for endosulfan I and II.
- From water samples stored at 24°C, alpha-, gamma-, and delta-BHCs showed only 19, 15, and 36 percent recovery, respectively.
- Heptachlor disappeared when stored at 24°C in the absence of chlorine.
- Endrin recovery was only 23 percent when water samples were adjusted to pH 2 and stored at 24°C.
- Endosulfan sulfate recovery was only 5 to 10 percent under all storage conditions investigated for spiked clean water samples. In addition, endosulfan sulfate did not disappear from wastewater after 7 days of storage. (A possible explanation given by the authors was the fact that the spiking level of endosulfan sulfate was increased 50 times in the case of wastewater.)

The stability of the organochlorine pesticides in soil has not been systematically investigated. Storage of soil samples at room temperature should be avoided since degradation of some organochlorine pesticides does occur (5). Losses of lindane and aldrin, after incubation of spiked soil samples at 25°C under aerobic conditions, were noticeable 7 days after the initiation of the experiment, and only 35 to 40 percent of the two pesticides were found at day 30 of the experiment (5).

Deep-freezing to -20°C appears to be the most suitable method for the storage of solid matrices since it has the widest range of application, causes the least changes in the samples, and makes the addition of preservatives unnecessary.

2.2 EXTRACTION

A number of methods have been used to extract PCBs and organochlorine pesticides from water, soil, and sediment samples. The extraction techniques that have been used on water samples include solvent extraction by stirring, separatory funnel partitioning, and liquid-liquid extraction; adsorption onto charcoal, polyurethane, and XAD-resins; and steam distillation. The methods that have been reported for soil and sediment samples include shaking, Soxhlet extraction, blending, and ultrasonication. Each of these techniques will be reviewed below.

TABLE A-2. MEAN RECOVERIES FROM PRESERVATION TESTS IN CLEAN WATER AFTER 7 DAYS AT 4°C[a]

Compound	Percent recovery		
	pH 2	pH 7	pH 10
Aldrin	89[b] (15)[c]	118[b] (12)[c]	86
alpha-BHC	64	60	82
beta-BHC	86	98	81
gamma-BHC	100	100	84
delta-BHC	80	108	74
4,4'-DDD	98	100	95
4,4'-DDE	94	89	92
4,4'-DDT	94	90	93
Dieldrin	105	107	102
Endosulfan I	96	103	0
Endosulfan II	102	98	0
Endosulfan sulfate	<10	<10	<10
Endrin	92	102	99
Endrin aldehyde	88	89	99[b] (0)[c]
Heptachlor	82	91	77[b] (401)[c]
Heptachlor epoxide	d	d	d
Chlordane	93	104	101
Toxaphene	103	101	120
PCB-1016	98	95	97
PCB-1221	98	92	93
PCB-1232	89	95	95
PCB-1242	95	88	98
PCB-1248	93	91	93
PCB-1254	99	97	95
PCB-1260	96	91	91

[a] Average of four replicates unless accompanied by parenthetical values. Data taken from Ref. 4.

[b] Average of two replicates.

[c] When residual chlorine was present; average of two replicates.

[d] Interference prevented assay.

TABLE A-3. MEAN RECOVERIES FROM PRESERVATION TESTS IN CLEAN WATER AFTER 7 DAYS AT 24°C[a]

	Percent recovery		
Compound	pH 2	pH 7	pH 10
Aldrin	91[b] (23)[c]	94[b] (15)[c]	89
alpha-BHC	65	68[b] (25)[c]	19
beta-BHC	84	87	95
gamma-BHC	94	93	15
delta-BHC	100[b] (64)[c]	101[b] (77)[c]	36[b] (26)[c]
4,4'-DDD	101	96	99
4,4'-DDE	97	89	117
4,4'-DDT	98	93	81
Dieldrin	95	101	106
Endosulfan I	104[b] (78)[c]	90	0
Endosulfan II	92[b] (80)[c]	81	0
Endosulfan sulfate	<10	<10	<10
Endrin	23	96	88[b] (119)[c]
Endrin aldehyde	94[b] (71)[c]	85	86[b] (0)[c]
Heptachlor	14	15[b] (84)[c]	10[b] (433)[c]
Heptachlor epoxide	d	d	d
Chlordane	97	98	103
Toxaphene	101	101	126
PCB-1016	96	99	93
PCB-1221	89	52[b] (94)[c]	86
PCB-1232	92	72[b] (85)[c]	96
PCB-1242	97	93	98
PCB-1248	90	93	92
PCB-1254	94	97	95
PCB-1260	92	90	86

---

[a] Average of four replicates unless accompanied by parenthetical values. Data taken from Ref. 4.

[b] Average of two replicates.

[c] When residual chlorine was present; average of two replicates.

[d] Interference prevented assay.

2.2.1 Extraction of Water Samples

Table A-4 is a summary of extraction methods published in the literature. The following describes in detail some of the procedures for the extraction of organochlorine pesticides and PCBs from water samples.

2.2.1.1 Stirring

This technique calls for stirring the sample with a nonpolar solvent in the original sample container. After stirring, the sample may then be transferred to a separatory funnel for further extraction. This method is particularly suited for aqueous samples with suspended material which can adhere to the walls of the sample container. Particles have been known to adsorb organochlorine compounds; their transfer to a separatory funnel is usually incomplete (1).

2.2.1.2 Separatory Funnel Partitioning

In this technique, up to 1 liter of aqueous sample is poured into a separatory funnel and is extracted by shaking with an organic solvent. The layers are allowed to separate, and the organic fraction is drawn off. Solvents with a specific gravity greater than 1 are preferred since the lower layer can be removed more easily.

The major drawbacks of this method are the limit of the sample size and the tendency of many wastewaters to form emulsions during extraction. To break emulsions, it has been recommended that the extract be passed through a 25-mm-thick glass-wool pad (27, 28).

Using separatory funnel partitioning on distilled water fortified with pesticides, Millar et al. (4) obtained the recoveries given in Table A-5. Although 15 percent methylene chloride in hexane gave slightly higher recoveries overall, methylene chloride alone settled to the bottom of the funnel and could be drained off more easily. Recoveries were greater than 80 percent under all conditions studied, except for aldrin and heptachlor. No reasons for the lower recoveries of these two pesticides were given. Method recoveries from two wastewater samples after cleanup are given in Table A-6. Lower recoveries in wastewater 2 may have been the result of a greater degree of emulsion formation or the result of erratic recovery from sulfur cleanup (4).

The American Society for Testing and Materials recommends that 1-liter water samples be extracted twice with 60 mL of ethyl ether:hexane (15:85). After saturating the solution with sodium sulfate, the sample is re-extracted with hexane. By using this procedure, recoveries ranging from 65 to 118 percent were obtained for aldrin, lindane, dieldrin, and DDT (29).

2.2.1.3 Liquid-Liquid Extraction

In this technique, several liquid-liquid extractors can be used in series to continuously extract an aqueous sample with an organic solvent.

TABLE A-4. EXTRACTION OF ORGANOCHLORINE PESTICIDES FROM WATER SAMPLES

Method	Solvent	Pesticides	Recovery (percent)	Concentration	Ref.
Activated carbon	Chloroform	Multi-OCS	75 to 86	2.5 to 5 ppm	6
Continuous	PE[a]	Aldrin, isodrin, dieldrin, endrin	95 to 100	7 to 340 ppb	7
Solvent (vortex)	Hexane and mixed solvents	Dieldrin, endrin	Average 61 to 95	0.2 to 0.5 ppb	8
Solvent (sep. funnel)	Benzene	Multi-OCS	90 to 100	0.06 to 1.2 ppb	9
RP L-L partition	PE	Multi-OCS	50 to 100	5 to 60 ppt	10
Solvent	Hexane	Toxaphene	75 to 85	0.25 to 1.25 ppm	11
Polyurethane foams	Hexane	Multi-OCS	87 to 100	0.5 to 1 ppb	12
Supported-bonded silicones	Pentane	Multi-OCS	--	10 to 20 ppt	13
Continuous	Cyclohexane, hexane	Multi-OCS	83 to 96	0.6 to 7.5 ppb	30
XAD-2	Acetonitrile	Multi-OCS	--	--	15
XAD-2	Diethyl ether	Multi-OCS	47 to 95	20 ppt	16
XAD-2, 4, 8, 12	10 percent ether in hexane	Multi-OCS	100 except aldrin 61	1 to 10 ppb	17
Polyurethane foams	Hexane	Multi-OCS	100	1 to 10 ppb	18
RP L-L partition	PE	Multi-OCS	73 to 107	1 to 10 ppb	19
XAD-2	Hexane	Multi-OCS	75 to 100	1.5 to 3 ppt	20
XAD-2	Diethyl ether	Multi-OCS	Average 96 distilled water Average 85 natural water except for mirex 52	0.01 to 0.1 ppb	21
Activated carbon	Chloroform	Multi-OCS	5 to 90	2 ppb	22
Solvent (sep. funnel)	Hexane	Multi-OCS	84 to 114	0.03 to 1.3 ppb	23
Solvent (sep. funnel)	Methylene chloride	Multi-OCS	74 to 104	0.09 to 66.5 ppb	24
Solvent (sep. funnel)	Benzene	Kepone	90 to 96	2 to 5 ppb	25
XAD-2, XAD-4, Tenax	Acetone, methanol, acetonitrile, n-pentane	Chlorinated hydrocarbons	--	ppt	26

[a] PE, petroleum ether.
OCS - organochlorine pesticides.

TABLE A-5. ORGANOCHLORINE PESTICIDES AND PCBs RECOVERIES BY EXTRACTION WITH METHYLENE CHLORIDE FROM WATER[a]

Compound	Percent recovery		
	pH 2	pH 7	pH 10
Aldrin (30)[b]	64[c]	46[d]	68[c]
alpha-BHC (20)	97	95	92
beta-BHC (40)	94	95	91
gamma-BHC (20)	109	92	101
delta-BHC (40)	104	92	97
4,4'-DDD (120)	97	97	93
4,4'-DDE (60)	94	92	81
4,4'-DDT (160)	102	104	92
Dieldrin (60)	96	100	101
Endosulfan I (50)	98	102	101
Endosulfan II (100)	98	99	94
Endosulfan sulfate (310)	98	92	89
Endrin (90)	106	108	108
Endrin aldehyde (230)	100	92	99
Heptachlor (20)	59	60	72
Heptachlor epoxide (40)	100	100	89
Chlordane (380)	103	99	102
Toxaphene (3800)	93	104	111
PCB-1016 (1290)	89	87	88
PCB-1221 (3000)	77	96	80
PCB-1232 (3000)	92	84	83
PCB-1242 (1500)	99	101	104
PCB-1248 (2250)	96	92	91
PCB-1254 (750)	91	101	96
PCB-1260 (1500)	101	100	100

[a] Data taken from Ref. 4.

[b] Amount in ng added to 1 liter of water.

[c] Average of three replicates.

[d] Average of four replicates.

TABLE A-6. RECOVERIES FROM WASTEWATERS (PERCENT OF SPIKED AMOUNT)[a]

Compound	Wastewater 1	Wastewater 2
Aldrin	93[b]	94[a]
alpha-BHC	102	82
beta-BHC	93	80
gamma-BHC	91	96
delta-BHC	98	79
4,4'-DDD	99	90
4,4'-DDE	99	84
4,4'-DDT	104	87
Dieldrin	97	94
Endosulfan I	97	95
Endosulfan II	110	84
Endosulfan sulfate	105	102
Endrin	95	95
Endrin aldehyde	95	86
Heptachlor	95	88
Heptachlor epoxide	99	91
Chlordane	96	86
Toxaphene	103	93
PCB-1016	92	95
PCB-1221	102	98
PCB-1232	100	86
PCB-1242	97	83
PCB-1248	99	87
PCB-1254	95	91
PCB-1260	95	91

[a] Data taken from Ref. 4.

[b] Average of three replicates.

This method has the advantages of being applicable to very large samples and of decreasing the likelihood of emulsion formation (26).

Using a 45-min petroleum ether extraction, Kahn and Wayman (7) obtained 95 to 100 percent recoveries of aldrin, isodrin, dieldrin, and endrin from wastewater. Using a modification of this liquid-liquid extraction and running at a pump rate of 52 L/hr, Ahnoff and Josefsson (30) obtained 89 to 96 percent extraction efficiencies for several organochlorine pesticides from water. Cyclohexane was chosen as the extracting solvent because of its greater phase separation from water. The amount of pesticides lost by adsorption to the glass reservoir walls was significant. For aldrin, which was the worst case, losses were 3.7 and 15.8 percent at 1.0 and 4.5 hours, respectively.

2.2.1.4 Steam Distillation

In this method, 2.5-liter water samples are heated to boiling. The steam is condensed and passed through a low-density solvent, such as isooctane or toluene, to extract chlorinated pesticides and PCBs.

According to Veith and Kiwus (31), the main advantage of this procedure is that it also serves as a cleanup method. Sample extracts may be directly injected onto the GC. Extraction recoveries for several PCBs and organochlorine pesticides by steam distillation are given in Table A-7.

2.2.1.5 Adsorption

Several techniques have been used to adsorb organochlorine pesticides and PCBs onto solid supports. The materials used include activated carbon (5, 22), support-bonded or coated polymers (13), polyurethane foams (12, 14, and 18), and polystyrene resins (15, 16, 17, 20, 21, 26). Once adsorbed from

TABLE A-7. RECOVERY OF PCBs AND PESTICIDES FROM WATER[a]

Compound	Quantity of chemical present (μg)	Steam-distillation recovery (percent)
PCB-1016	0.04[b]	97.9
PCB-1242	0.04	99.4
PCB-1248	0.04	100.0
PCB-1254	0.04	99.1
4,4'-DDE	0.02	104.0
Heptachlor epoxide	0.01	89.9
Mirex	0.04	89.5

[a] Data taken from Ref. 31.

[b] Quantity added to water, individual analyses.

water, pesticides and PCBs may be desorbed by either elution or Soxhlet extraction with an organic solvent such as chloroform (22), petroleum ether or pentane (26), or hexane (18). While many of these methods work well for distilled water fortified with pesticides, recoveries are often poor for wastewaters. Furthermore, adsorption sites on these resins can become deactivated by impurities in natural waters (1).

2.2.2 Extraction of Sediment and Soil Samples

This section summarizes the extraction techniques reported in the literature for soil and sediment samples. A detailed discussion of the extraction of organochlorine pesticides from soil was published by Chiba (32) and will be summarized below. Examples of extraction techniques, solvent and solvent mixtures used for extraction, and recovery data are presented in Table A-8.

Techniques used for the extraction of organochlorine pesticides from soil can be classified into four major groups: (a) surface rinsing by rotating or tumbling, (b) blending with solvents, (c) Soxhlet extraction, and (d) sonication. Each of these techniques will be described in detail below. After the description of extraction techniques, other factors that affect the extraction recovery such as solvent(s), contact time between the solvent and the solid matrix, and soil factors (e.g., moisture) will be addressed.

2.2.2.1 Extraction Equipment

Tumbling and Shaking

Several types of tumblers including end-over-end tumbler, rotary shaker, Northcott shaker, head-to-end tumbler, mechanical shaker, and wrist-action shaker have been used for pesticide residue determination.

The tumbling techniques have the advantage of giving a smaller amount of coextractants than other methods, but the recoveries may vary greatly between laboratories (1 and 32).

Woolson (48) used a 1:1 solution of hexane and acetone to extract soil premoistened with 0.2M NH_4Cl. Recoveries from spiked samples averaged 92 percent with a 13.58-percent coefficient of variation (Table A-9).

Wheatly (49) reported recoveries between 51 and 64 percent for dieldrin when the tumbling technique was used with hexane:2-propanol or hexane:acetone.

Lichtenstein (50) used the tumbling method and reported recoveries of 92 to 98 percent for DDT, 92 to 97 percent for aldrin, 90 to 94 percent for dieldrin, and 100 percent for lindane.

TABLE A-8. EXTRACTION OF ORGANOCHLORINE PESTICIDES FROM SOIL AND SEDIMENT SAMPLES

Method	Sample	Solvent	Pesticides	Recovery (percent)	Level	Ref.
Ultrasonic	Soil	Acetone	Multi-OCS	94 to 104	2 ppm	33, 34 35
Blender	Soil	21 solvent systems	Aldrin, Dieldrin	--	0.1 to 0.9 ppm	36
Soxhlet	Soil	41:59 hexane:acetone	Multi-OCS	--	0.01 to 0.4 ppm	37
Soxhlet	Soil	41:59 hexane:acetone	Multi-OCS	95 to 108	0.25 to 6.5 ppm	5
Blender	Soil	Methylene chloride	Multi-OCS	22 to 155	0.4 and 4.0 ppm	38
Shaker	Soil	3 solvent systems	Multi-OCS	Average 74.6	0.5 to 6.0 ppm	39
Soxhlet	Soil	5 solvent systems	Multi-OCS	Average 87	0.5 to 6 ppm	39
Multi	Soil	Multi	Dieldrin	--	--	40
Shaker	Soil	1:1 hexane:acetone[a]	Chlordanes and others	--	2 to 4 ppm	41
Blender	Soil	1:1 hexane:acetone	Chlordanes and others	--	2 to 4 ppm	41
Soxhlet	Sediments	41:59 hexane:acetone	Toxaphene	Average 88	0.25 ppm	11
Shaker	Sediments	2:1 and 4:1 hexane:acetone	Multi-OCS	75 to 99	0.5 to 1 ppb	42
Shaker	Sludge	1:2 hexane:acetone	OCS and PCBs	--	OCS = 0.005 to 0.24 ppm	43
Rotation	Sediment	Acetone; 1:3 acetone:hexane	OCS and PCBs	OCS = 73 to 97	OCS = 50 ppb	44
Soxhlet	Soil	1:9 acetone:PE	OCS and PCBs	OCS = 82 to 112	OCS = 1.3 to 3.3 ppb	45
Column	Sediment	1:1 hexane:acetone	Multi-OCS	--	--	27
Column	Soil	4:3:3 hexane: acetone:methanol	4,4'-DDT	78 to 87	10 ppm	46
Soxhlet	Sediment	35:65 ether:hexane	Kepone	103	4 ppm	47

[a] Other solvents evaluated include: hexane:2-propanol (1:1); methanol; benzene:methanol (1:1).

OCS - organochlorine pesticides.

PE - petroleum ether.

TABLE A-9. RECOVERIES OF ORGANOCHLORINE PESTICIDES ADDED TO CHRISTIANA CLAY LOAM[a]

Compound	Added (ppm)	Average Recovery (ppm)	Average Recovery (percent)	Standard deviation (ppm)	Coefficient of variation (percent)
Lindane	0.94	0.81	86	0.08	9.35
Heptachlor	1.52	1.32	87	0.20	15.25
Aldrin	0.98	0.84	86	0.11	13.12
Heptachlor epoxide	0.49	0.49	100	0.07	13.80
4,4'-DDE	0.82	0.79	96	0.06	7.62
Dieldrin	1.56	1.42	91	0.15	10.45
Endrin	1.17	1.09	93	0.13	11.65
4,4'-TDE	1.35	1.25	97	0.20	16.03
2,4'-DDT	3.64	3.28	90	0.84	25.47
4,4'-DDT	4.27	3.82	89	0.48	12.69
Average of all data			92	0.23	13.58

[a] Data taken from Ref. 48.

The sample size used in tumbling extraction was generally larger than that used in shaking extraction; for example, most workers used 100 to 500 g for tumbling and 10 to 200 g for shaking.

Blending

In this technique, soil samples are blended with solvent for a relatively short period of time which is generally 2 to 15 min. The method is very popular for the extraction of plant materials but is less widely used with soils. Sample sizes of 25 to 100 g are generally required (1 and 32).

Several types of blenders are available commercially; the most commonly used are Waring and Omni-Mix blenders. Lichtenstein (50) used a Volu-Mix Lourdes homogenizer at medium speed for 5 min for 75 g soil and 150 mL hexane: acetone (1:1). Chiba and Morley (36) used an Omni-Mix high-speed blender and found that it was necessary to repeat the 5-min extraction with the same solvent. This was done not only to achieve complete extraction but also to minimize loss due to pesticide readsorption during filtration.

In a comparison of the efficiencies of tumbling, Soxhlet extraction, and blending at equal extraction times, blending was the most efficient technique (32). When acetonitrile was used as the extracting solvent, recoveries of several organochlorine pesticides and PCBs were over 95 percent (51). Another study (36) compared blender extraction efficiency on aldrin and dieldrin. The extraction solvents dimethyl formamide, dimethyl sulfoxide, and acetone gave the best results while the results with methylene chloride, n-hexane, and propylene carbonate were poor (36).

Soxhlet Extraction

This technique has been commonly used for extracting pesticides from soil. The main advantage of this technique is the ease and neatness in handling. Because of the nature of the system, air-dried soil is preferred; however, wet soil has also been extracted in a Soxhlet by using water-miscible solvents (28).

Chiba and Morley (36) reported that the Soxhlet extraction method was very efficient with most solvent systems although the amounts of coextractants were always higher when compared to the blending technique (Table A-10). The authors concluded that there is little advantage in using the Soxhlet procedure since a stringent cleanup method is required to remove the large amount of coextractants. Furthermore, they reported that it is difficult to obtain good reproducibility because of the difficulty in keeping the solvent-recycle time constant. Acetonitrile gave the fastest rate of extraction while n-hexane:acetone gave the slowest. Despite the fact that n-hexane:acetone has a slow solvent-recycle time, the use of hexane:acetone (41:59) which forms an azeotrope with a boiling point of 50°C decreases the chance of pesticide degradation (1, 37). When acetonitrile (boiling point 82°C) was used, for example, DDT degraded to DDE (37).

By using Soxhlet extraction with hexane:acetone, it was determined that the moisture content of the soil could affect extraction efficiency. At least 5 percent water was essential for good recoveries, and up to 40 percent water had no detrimental effect (37).

Ultrasonication

This technique uses an ultrasonicator to extract pesticides into an organic solvent. It has the advantage of providing quantitative recoveries in short periods of time. It may also be particularly useful for the extraction of "aged" samples because of its ability to break up cells and soil colloids (34).

With acetone, a 30-sec ultrasonication gave higher recoveries than the roller or blender methods and was better than or equal to an 8-hour Soxhlet extraction (Table A-11) (35).

2.2.2.2 Solvents

Several solvents and solvent mixtures have been used for the extraction of organochlorine pesticides from soil. These include: petroleum ether (52), hexane (52), pentane (52), hexane:acetone (5, 37, 41, 42, 43, 44, 27), acetone (33, 34, 35), acetonitrile (54), propylene carbonate (59), benzene

TABLE A-10. COMPARISON OF EXTRACTION EFFICIENCIES[a]

Extraction No.	Solvent	Method	Coextractants (mg/g)	Aldrin (ppm)	Dieldrin (ppm)
13	50 percent acetone:n-hexane	B 5 x 2	0.085	0.51	0.47
22	50 percent acetone:n-hexane	19 B 5 x 2	0.097	0.83	0.87
23	50 percent acetone:n-hexane	B 1 hr x 1	0.150	0.88	0.93
24	50 percent acetone:n-hexane	Soxhlet, 12 hr	0.340	0.90	0.87
25	50 percent acetone:n-hexane	B 5 hr x 1	0.190	0.92	0.89
9	Dimethylformamide	B 5 x 2	0.022	0.92	0.87
26	Dimethylformamide	B 2 hr x 1	0.038	0.88	0.95
27	Dimethylformamide	19 B 5 x 2	0.048	0.88	1.02
28	Dimethylformamide	Soxhlet, 20 hr[b]	0.470	0.99	1.08
29	Methanol	19 B 5 x 2	0.043	0.90	1.04
30	Methanol	Soxhlet, 12 hr	0.180	1.01	1.06
31	50 percent methanol:benzene	19 B 5 x 2	0.320	0.97	0.95
32	50 percent methanol:benzene	Soxhlet, 12 hr	0.490	0.92	1.07
33	Acetonitrile	19 B 5 x 2	0.033	0.72	0.66
34	Acetonitrile	Soxhlet, 12 hr	0.200	1.00	1.05
35	Acetone	19 B 5 x 2	0.045	0.86	0.93
36	Acetone	Soxhlet, 12 hr	0.062	0.89	0.90
37	Benzene	19 B 5 x 2	0.077	0.49	0.41
38	Benzene	Soxhlet, 12 hr	0.570	0.81	0.86
39	50 percent 2-propanol:n-hexane	Soxhlet, 12 hr	0.230	0.62	0.77
40	50 percent acetone:benzene	19 B 5 x 2	0.150	0.61	0.66
41	Methylene chloride	Soxhlet, 12 hr	0.220	0.18	0.48

[a] Data taken from Ref. 36.

[b] Temperature of oil bath adjusted so that 5 min were required to finish one extraction cycle.

Note:

- B 5 x 2. Air-dried soil (100 grams) and the extraction solvent (200 mL) were blended for 5 min with external cold-water cooling. The mixture was filtered with suction by using Whatman No. 1 filter paper with a 1-cm pad of SuperCel. The container was rinsed with 2 x 25 mL of the solvent; the soil, filter paper, and pad were returned to the blender, and the extraction procedure was repeated.

- B 5 x 1. The B 5 x 2 procedure was modified by adding anhydrous Na_2SO_4 or by using moist soil in experiments where comparisons with the standard method were being made. The extraction was done just once; 4 x 50 mL of the solvent were used for rinsing instead of 2 x 25 mL.

- 19 B 5 x 2. To check the influence of the contact time between solvent and soil, soil (100 grams) was kept in contact with solvent in a container for 19 hours (overnight) prior to carrying out the standard procedure.

- Soxhlet. A soil-solvent ratio of 1:5 was used, and the steam bath was adjusted so that it took 5 min to complete one extraction cycle with methanol (boiling point 65°C). In the case of mixed solvents, soil extraction was actually made by the azeotropes, but the normal 1:1 ratio of solvents was added to the flask initially so that working-up procedures could be kept consistent.

- B 1 hr x 1, B 2 hr x 1, and B 5 hr x 1 are identical to B 5 x 2 except that the blending time was extended to 1 hr, 2 hr, and 5 hr, respectively.

TABLE A-11. COMPARISON OF EXTRACTION METHODS FOR EFFICIENCY OF EXTRACTION OF HEPTACHLOR EPOXIDE AND DIELDRIN FROM A WET AND DRY CLAY SOIL USING ACETONE[a]

Extraction method	Soil moisture	Mean percent recovery and deviation from mean Heptachlor epoxide	 Dieldrin
Ultrasound	Dry	97.6 ± 0.4	98.3 ± 0.0
	Wet	99.3 ± 0.1	96.0 ± 1.1
Blender	Dry	84.2 ± 2.7	79.3 ± 2.7
	Wet	95.2 ± 0.6	93.1 ± 0.3
Roller	Dry	87.8 ± 1.1	85.8 ± 0.9
	Wet	93.7 ± 0.1	89.7 ± 0.1
Soxhlet	Dry	86.5	82.0
	Wet	99.8 ± 1.9	97.0 ± 2.4

[a] Data taken from Ref. 35.

(36), pentane:2-propanol:glacial acetic acid (4:1:1) (55), benzene: 2-propanol (56), n-hexane:2-propanol (56), pentane:acetone (56); chloroform: methanol (1:1) (57), and methylene chloride:methanol (1:1) (58). Observations made by the various authors referenced above include:

- Petroleum ether has been recommended for most organochlorine compounds because the solvent will give satisfactory clean extracts (52).
- n-hexane:acetone (4:1) extracted 10 to 20 percent more aldrin than hexane alone (53).
- Although mixtures of n-hexane:acetone appear to be fairly efficient, the best mixing ratio has not been determined, and ratios varying from 1:1 to 19:1 have been used (1).
- Acetonitrile was used primarily for vegetables and fruits. Duffy and Wong (53) evaluated this solvent for a number of soils and found that it was less efficient than a mixture of n-hexane and 2-propanol (3:1).
- In general, improved extraction efficiency was achieved when two different solvent systems were combined (60). However, this was only advantageous when the solvent was the only factor controlling the extraction efficiency. It was possible to achieve satisfactory extraction with one solvent by changing the moisture content of the sample, the contact time between solvent and soil, and the type of extractor.

Twenty-one solvent systems were investigated by Chiba and Morley (36) for the extraction of aldrin and dieldrin from different types of soils (Table A-12). Of the nine single-solvent systems, dimethylformamide (DMF) showed the best results, followed by dimethyl sulfoxide (DMSO) and acetone. Benzene, methylene chloride, n-hexane, and propylene carbonate gave poor results, and methanol gave good recovery for dieldrin only. Furthermore, most polar solvents tended to give lower amounts of coextractants whereas the nonpolar solvents did the opposite. No correlation between the concentration of the pesticides and the amount of coextractants was found.

Williams compared the extraction efficiency of three solvent systems using mechanical shaking (37). With one soil, hexane:isopropanol gave highest recoveries while with another one, hexane:acetone was superior. In a measure of the extraction of technical chlordane from aged soil samples when the mechanical shaker method was used, recovery decreased in the order methanol>ethanol>hexane:acetone>hexane:isopropanol>acetone (38).

Johnsen and Starr (35) studied the extraction efficiency of six different solvent systems for the extraction of dieldrin and heptachlor epoxide from sandy loam soil by using three different methods over three time intervals. The solvent systems investigated were acetonitrile, water-saturated benzene, benzene:2-propanol (2:1), benzene:methanol (2:1), n-hexane:2-propanol (2:1), and n-hexane:acetone (4:1). They reported that benzene was a poor solvent, that benzene:methanol gave the highest average recoveries for all three extraction times, and that n-hexane:acetone gave the highest recoveries for all three methods when the sample was extracted one day after spiking with the pesticides, but that the efficiency of this solvent system decreased significantly when the sample was extracted one month after spiking.

Residues of chlordane were extracted from clay loam soil (38) with different solvents under various conditions; the results indicate that mixtures of hexane:acetone gave better recoveries than hexane:2-propanol and that acetone alone gave poor recoveries. The use of the hexane:acetone mixture increased the efficiency of extraction, but there was no significant improvement in efficiency by increasing the proportion of hexane in acetone from 50 to 90 percent. Furthermore, methanol gave higher recoveries than any other solvent or mixture of solvents.

2.2.2.3 Time

The time factor was investigated by Chiba and Morley (36). As shown in Table A-10, the time factor was very important when the blending method was used. Only 0.51 ppm aldrin and 0.47 ppm dieldrin were extracted when the standard contact time was used, but all other modified methods showed nearly double the amount of each pesticide. The 19-hour contact time with the solvent, which was followed by normal blending, was one of the most promising methods because the recovery of pesticides was high, and the amount of coextractants was low. There appeared to be no advantage in extending the blending time to more than 1 hour.

Goerlitz and Law (42) have performed five separate extractions of fortified sediment samples with acetone/hexane and have reported 97.9 percent average

TABLE A-12. COMPARISON OF EXTRACTION EFFICIENCIES OF SOLVENTS[a]

Extraction number	Solvents	Co-extractants, (mg/g)	Aldrin (ppm)	Dieldrin (ppm)
1	Methylene chloride	0.079	0.10	0.30
2	Methanol	0.032	0.10	0.55
3	Propylene carbonate	0.016	0.17	0.09
4	n-Hexane	0.110	0.21	0.19
5	Benzene	0.069	0.33	0.29
6	Acetonitrile	0.025	0.50	0.45
7	Dimethylsulfoxide (DMSO)	0.011	0.74	0.80
8	Acetone	0.032	0.81	0.73
9	Dimethylformamide (DMF)	0.022	0.92	0.87
10	50 percent acetone:methylene chloride	0.140	0.30	0.38
11	50 percent 2-propanol:n-hexane	0.076	0.33	0.32
12	10 percent acetone:n-hexane	0.071	0.45	0.39
13	50 percent acetone:n-hexane	0.085[b]	0.51[b]	0.47[b]
14	50 percent acetonitrile:acetone	0.061	0.57	0.51
15	50 percent acetone:benzene	0.130	0.60	0.44
16	50 percent acetonitrile:n-hexane	0.040	0.60	0.53
17	50 percent acetonitrile:benzene	0.086	0.61	0.55
18	50 percent DMSO:benzene	0.190	0.79	0.72
19	50 percent methanol:methylene chloride	0.180	0.82	0.72
20	50 percent methanol:benzene	0.240	0.85	0.85
21	50 percent DMF:benzene	0.170	0.89	0.87

[a] Extraction method: Omni-Mix high speed blender, two 5-min extractions with 200 mL of solvent per 100 grams of soil (B 5 X 2). Data taken from Ref. 36.

[b] Standard deviation: aldrin ±0.03; dieldrin ±0.02 (eight determinations). Soil 1 (sandy loam), air-dried.

recoveries of DDE, DDT, DDD, lindane, and PCB from five different clay-type sediments and from five silt materials (Table A-13).

2.2.2.4 Moisture

Chiba and Morley (36) tried to determine the role of sodium sulfate in conjunction with the moisture content of soil. Table A-14 shows the significance of moisture content when use is made of the 50-percent acetone:n-hexane system with both blending and tumbling methods. The difference in extraction efficiency between air-dried soil and soil with a moisture content of 25 percent was significant when sodium sulfate was not added. When sodium sulfate

TABLE A-13. EXTRACTION OF CONTAMINATED BOTTOM MATERIAL[a]

		Extraction					
		1 + 2 + 3		4[b]		5[c]	
Sample	Compound	(ng)	(percent)	(ng)	(percent)	(ng)	(percent)
Clay 1	DDE	88.0	99.3	0.00	0.0	0.64	0.7
	DDD	132	100.0	0.00	0.0	0.00	0.0
	DDE	60.9	100.0	0.00	0.0	0.00	0.0
Silt 1	DDE	85.4	98.4	0.57	0.7	0.78	0.9
	DDD	127	100.0	0.00	0.0	0.00	0.0
	DDT	79.5	99.1	0.00	0.0	0.76	0.9
Clay 2	DDE	153	98.2	1.75	1.1	1.13	0.7
	DDD	266	97.6	4.16	1.5	2.34	0.9
	DDT	454	98.6	1.91	0.4	4.31	0.9
Silt 2	DDE	319	97.1	3.58	1.1	6.05	1.8
	DDD	542	98.3	3.83	0.7	5.35	1.0
	DDT	1,050	98.5	5.21	0.5	10.5	1.0
Clay 3	Lindane	724	90.9	22.1	2.8	50.3	6.3
	DDE	4,530	98.4	18.8	0.4	55.3	1.2
	DDD	11,100	98.4	57.4	0.5	128	1.1
	DDT	16,500	97.2	133	0.8	347	2.1
Silt 3	Lindane	1,030	83.8	54.9	4.5	145	11.8
	DDE	7,680	98.7	22.3	0.3	82.7	1.1
	DDD	18,500	99.0	56.8	0.3	136	0.7
	DDT	24,400	98.2	123	0.5	333	1.3
Clay 4	DDE	802	99.5	1.87	0.2	2.35	0.3
	DDD	990	98.3	5.97	0.6	11.5	1.1
	DDT	1,400	98.4	9.03	0.6	13.1	0.9
Silt 4	DDE	601	99.0	2.37	0.4	3.50	0.6
	DDD	1,050	98.5	6.15	0.6	9.64	0.9
	DDT	1,070	98.5	9.23	0.9	7.61	0.7
Clay 5	PCB	3,530	99.9	1.59	0.0	0.89	0.0
Silt 5	PCB	5,090	100.0	0.16	0.0	1.50	0.0
Average			97.9		0.7		1.4

[a] Data taken from Ref. 42; the extraction solvent was acetone/hexane.
[b] Extract from fourth extraction analyzed separately.
[c] Fifth extraction performed after 18-hour exposure to acetone.

TABLE A-14. INFLUENCE OF MOISTURE CONTENT

Extraction number	Moisture content (percent)	50 g of Na_2SO_4	Method	Co-extractants (mg/g)	Aldrin (ppm)	Dieldrin (ppm)
Soil 1						
13	3.1[a]	-	B 5 x 2	0.085	0.51	0.47
45	3.1	+	B 5 x 2	0.260	0.41	0.41
46	3.1	-	Tumbler	0.044	0.42	0.37
47	3.1	+	Tumbler	0.066	0.50	0.39
48	25[b]	-	B 5 x 2	0.130	0.95	1.08
49	25	+	B 5 x 2	0.120	0.40	0.45
50	50	-	B 5 x 2	0.190	0.82	1.00
51	50	+	B 5 x 2	0.120	0.81	0.95
52	50	-	Tumbler	0.120	0.84	1.08
53	50	+	Tumbler	0.100	0.72	0.71
54	75	-	B 5 x 2	0.140	0.95	1.04
55	75	+	B 5 x 2	0.180	1.00	1.08
56	100	-	B 5 x 2	0.120	0.85	1.08
57	100	+	B 5 x 2	0.140	0.95	0.96
Soil 2						
58	1.8[a]	-	B 5 x 2	0.088	2.35	0.90
59	1.8	+	B 5 x 2	0.110	1.20	0.40
60	1.8	-	Tumbler	0.072	1.25	0.45
61	1.8	+	Tumbler	0.110	1.55	0.50
62	16.7[c]	-	B 5 x 2	0.310	2.42	1.24
63	16.7	+	B 5 x 2	0.910	2.59	1.18
64	16.7	-	Tumbler	1.830	2.59	1.30
65	16.7	+	Tumbler	1.050[d]	2.59	1.18

[a] Air-dried.

[b] Water added up to 25, 50, 75, and 100 percent of water-holding capacity.

[c] Natural field moisture content.

[d] Extract showed orange color, probably from rubber gasket material on the Mason jar. Soil type: sandy loam. Extraction solvent: 50 percent acetone:n-hexane. Data taken from Ref. 36.

was added, the most significant difference was found between 25 and 50 percent moisture content. The authors also concluded that there may be some risk involved in the use of sodium sulfate unless the moisture content is measured precisely. When the moisture content is less than 25 percent, the use of sodium sulfate may reduce the extraction efficiency.

Williams (37) investigated the effect of water content on Soxhlet extraction with two silt loam soils. The results were consistent for samples with a moisture content between 5 percent and 40 percent and were different from the results obtained with the dry soils (Table A-15).

Saha (41) studied the effect of the addition of 20 percent water to air-dried soil before extraction and reported considerably increased extraction efficiencies for methanol, hexane:acetone, hexane:2-propanol, and benzene:methanol. The amount of water added to the soil before extraction with hexane:acetone (1:1) resulted in increased recoveries; however, addition of 40 to 50 percent water reduced the recovery of chlordane residues, and 100 percent water gave poor recoveries (Table A-16).

2.3 EXTRACT CLEANUP

Several types of cleanup techniques are available for the removal of coextractants from a sample matrix. They are listed below:

- Liquid-liquid partitioning
- Gel permeation chromatography
- Sulfur removal
- Liquid-solid chromatography (alumina, Florisil, silica gel)
- High-pressure liquid chromatography (HPLC).

Each of these techniques will be discussed in the following sections.

2.3.1 Liquid-Liquid Partitioning

This technique is widely used for removing fats, waxes, and polar materials. The sample extract is, for example, partitioned with a solvent of different polarity which is immiscible with the extraction solvent. Thus, if the extract is in hexane, petroleum ether, or benzene, it will be partitioned with a polar solvent such as acetonitrile. The waxes and fatty materials will remain in the nonpolar phase while the pesticides will partition into the acetonitrile. The polar phase containing the pesticides is diluted with water, and a sodium sulfate or sodium chloride solution is added to "salt out" the pesticides from the polar phase back into another nonpolar solvent. Recovery data for solvent partition of pesticides between one part of hexane and three parts of polar solvent are given in Table A-17.

Solvent systems such as DMF:hexane (62), DMSO:petroleum ether (63), and acetone:petroleum ether have been recommended for partitioning to separate organochlorine pesticides from fat. Although DMF and DMSO were superior in terms of recovery, and DMSO was the best in removing fat, these two solvent systems have not been widely used because of emulsion problems (1).

TABLE A-15. EFFECT OF WATER ON THE RECOVERY IN PPM OF ORGANOCHLORINE PESTICIDES FROM TWO SAMPLES OF SILT LOAM SOIL BY SOXHLET EXTRACTION[a]

Pesticide	Water added (percent) 0	5	10	20	30	40
Soil 1						
Dieldrin	0.15	0.18	0.19	0.20	0.20	0.20
4,4'-DDE	0.054	0.058	0.062	0.068	0.068	0.064
2,4'-DDT	0.023	0.032	0.032	0.027	0.024	0.021
4,4'-DDT	0.37	0.41	0.41	0.40	0.40	0.40
Soil 2						
Heptachlor	0.007	0.008	0.008	0.008	0.009	0.009
Heptachlor expoxide	0.18	0.24	0.25	0.26	0.24	0.24
4,4'-DDE	0.036	0.042	0.044	0.042	0.041	0.042
4,4'-DDT	0.031	0.043	0.047	0.047	0.047	0.045
4,4'-DDT	0.25	0.34	0.35	0.35	0.36	0.36

[a]Data taken from Ref. 37.

TABLE A-16. EFFECT OF THE AMOUNT OF WATER ADDED TO AN AIR-DRIED CLAY LOAM SOIL ON THE RECOVERY OF CHLORDANE RESIDUES WITH 1:1 HEXANE:ACETONE (MECHANICAL SHAKER METHOD)[a]

Water added to soil (percent)	Recovery (ppm) cis-Chlordane	trans-Chlordane	Others	Total
0.0	0.58	0.68	1.38	2.64
10.0	0.78	0.92	2.08	3.78
20.0	0.88	0.93	2.12	3.93
30.0	0.88	0.93	2.10	3.91
40.0	0.84	0.90	1.98	3.72
50.0	0.75	0.88	1.88	3.51
100.0	0.36	0.41	0.89	1.66

[a]Data taken from Ref. 41.

TABLE A-17. SOLVENT PARTITION OF PESTICIDES AND BUTTER OIL BETWEEN 1 PART HEXANE AND 3 PARTS POLAR SOLVENT[a]

Pesticide	Methanol	Acetonitrile	DMSO	DMF
Aldrin	73	50	49	74
Butter oil	17	7.1	0.3	11
DDE	69	56	60	93
DDT	77	68	96	97
Dieldrin	75	74	85	93
Endrin	84	87	95	96
Heptachlor	83	54	80	92
Heptachlor epoxide	90	88	97	97
Lindane	89	96	98	98

[a] Data taken from Ref. 61.

2.3.2 Gel Permeation Chromatography

In gel permeation chromatography, the separation mechanism is based on differences in molecular size. Large molecules are excluded from the pores of the gel and elute first while smaller molecules which can diffuse into the pores are eluted last.

Gel permeation chromatography with Bio-Beads SX-2, SX-3 combined with cyclohexane, and cyclohexane-methylene chloride as eluants were reported for the cleanup of fish lipids (64). Other mobile phases included toluene:ethyl acetate (1:3 v/v) and ethyl acetate.

Bio-Beads SX-3 and toluene:ethyl acetate have been used successfully to remove lipid interferences from fish extracts for subsequent determination of Kepone (27).

The elution volumes of organochlorine pesticides and PCBs obtained from Bio-Beads SX-3 when two different solvent mixtures are used are given in Table A-18.

2.3.3 Sulfur Removal

Sulfur and organosulfur compounds, if present, will give large peaks in the solvent area which often mask the region from the solvent peak to the aldrin peak.

Several methods are available for the removal of sulfur. Shaking with 0.1 to 0.2 mL of metallic mercury (65) is a simple technique, but quite often it does not remove all the sulfur. Although Goerlitz and Law (65) claimed that aldrin, dieldrin, heptachlor, lindane, and 4,4'-DDT were not affected by

TABLE A-18. ELUTION VOLUMES OF ORGANOCHLORINE PESTICIDES BY GEL PERMEATION CHROMATOGRAPHY[a]

Organochlorine pesticides	Elution volume (mL) with methylene chloride: cyclohexane (15:85)	Elution volume (mL) with toluene:ethyl acetate (1:3)
Aldrin	110->150	110->130
alpha-BHC	150->190	90->130
alpha-Chlordane	120->170	100->120
gamma-Chlordane	120->170	100->120
4,4'-DDD	160->210	90->120
4,4'-DDE	110->160	110->160
2,4'-DDT	110->150	110->130
4,4'-DDT	120->170	110->130
Dieldrin	140->180	110->130
Endrin	130->170	110->130
Heptachlor	110->150	100->130
Heptachlor epoxide	120->170	110->130
Hexachlorobenzene (HCB)	120->160	120->150
Lindane	160->210	100->130
Methoxychlor	140->200	100->120
Mirex	90->140	110->130
Toxaphene	120->240	100->140
PCBs and PBBs		
PCB-1016	120->240	NT
PCB-1242	120->240	110->140
PCB-1254	120->240	100->150
PCB-1260	120->240	110->140
Kepone	NT	100->190

NT -- Not tested.

[a] Data taken from Ref. 66.

the addition of Hg to the hexane solutions of these pesticides, Thompson (27) reported that recovery of heptachlor was only 39.8 percent when Hg was used for the removal of sulfur (Table A-19).

Activated copper (copper dust treated for removal of surface oxides with 2N HCl) was also used (67). Heptachlor was reported to be degraded by copper activated with 6 N nitric acid (27); Chau et al. (1) found no degradation for 17 organochlorine pesticides dissolved in isooctane when contacted with HCl-activated copper for 7 days.

TABLE A-19. EFFECT OF EXPOSURE OF ORGANOCHLORINE PESTICIDES TO VARIOUS SULFUR REMOVAL REAGENTS

	Percent recovery					
Pesticide	Hg[a]	Cu[a]	H_2SO_4[b]	H_2SO_4/KCN[b]	KOH[b]	TBA[c]
Aldrin	95.5	93.3	92	87	104	73
PCB-1254	97.1	104.3	--	--	--	--
alpha-BHC	81.2[d]	98.1[d]	94	59	0	--
beta-BHC	--	--	94	70	6	--
gamma-BHC (lindane)	75.7	94.8	92	55	11	79
4,4'-DDD	--	--	100	95	0	94
4,4'-DDE	92.1	102.9	104	100	437[e]	95
4,4'-DDMV[f]	--	--	100	91	190[e]	--
4,4'-DDT	79.8d	85.1[d]	100	95	0	94
2,4'-DDT	--	--	98	95	0	--
Dieldrin	79.1	94.9	0	0	103	--
Endrin	90.8	89.3	--	--	--	--
Heptachlor	39.8	5.4	94	90	104	--
Heptachlor epoxide	69.1	96.6	83	79	100	97

[a] Ref. 27, level not specified, recovery based on mean of duplicate tests. Copper dust activated with 6N HNO_3.

[b] Ref. 43, level 0.02 to 0.1 ppb.

[c] Ref. 45, level 50 ppb, TBA is tetrabutyl ammonium sulfite.

[d] Isomer not specified in original literature.

[e] High recovery is due to the formation of pesticide by the decomposition of other.

[f] 4,4-DDMV is 1-chloro-2,2-bis(4-chlorophenyl)ethane.

Ahnoff and Josefsson (68) showed that sulfur was easily removed by shaking with Raney Nickel or KOH at 120°C for 20 min. This treatment did not affect the PCBs; however, DDT and DDD were converted to DDE and DDMV [1-chloro-2,2-bis(4-chlorophenyl)ethane], respectively, and most BHCs were lost.

An efficient, rapid, nondestructive procedure to remove sulfur according to the reaction:

$$(TBA^+)_2SO_3^{2-} + S \text{ ------> } 2TBA^+ + S_2O_3^{2-}$$

where TBA is the tetrabutyl ammonium ion was reported by Jensen et al. (44). Results of recovery experiments from spiked sediments are reported in Table A-20.

TABLE A-20. RESULTS OF RECOVERY EXPERIMENTS FROM SPIKED SEDIMENTS USING TETRABUTYL AMMONIUM SULFITE[a]

Substance	n	True value (ppb)	Mean (percent)	Standard deviation, percent of mean value
4,4'-DDE	7	50	95	7.2
4,4'-DDD	7	50	94	6.4
4,4'-DDT	7	50	94	5.7
2,3',4',5-Tetrachlorobiphenyl[b]	7	50	97	5.5
2,2',3',4,5,6-Hexachlorobiphenyl[b]	6	2.8	100	4.0
2,2',4,4',5,5'-Hexachlorobiphenyl[b]	7	50	98	5.4
2,3,3',4,4'-Pentachlorobiphenyl[b]	6	2.8	95	4.0
2,2',3,4,4',5'-Hexachlorobiphenyl[b]	6	2.8	101	9.1
Hexachlorobenzene	7	50	94	3.4
Lindane (gamma-BHC)	7	50	79	4.4
Aldrin	7	50	73	5.4
Aldrin, prior to evaporization of extracting solvents	4	50	95	1.9
Heptachlor epoxide	7	50	97	1.2
2,4,6-Trichlorophenol	4	20	75	2.8
2,3,4,6-Tetrachlorophenol	4	20	82	1.8
Pentachlorophenol	4	50	101	1.9

n - number of replicates.
[a]Data taken from Ref. 44.
[b] - representative PCB component.

2.3.4 Liquid-Solid Chromatography

The following discussion will emphasize the application of Florisil, alumina, and silica gel to the cleanup and fractionation of organochlorine pesticides and PCBs in extracts.

2.3.4.1 Florisil

Florisil is a synthetic magnesium silicate manufactured by the Floridin Company from magnesium sulfate and sodium silicate. Following precipitation from solution, it is filtered, dried, and calcinated at 650°C. Examples of cleanup methods for organochlorine pesticides and PCBs using Florisil are listed in Table A-21.

Mills (69) introduced the use of Florisil in the cleanup of nonfatty and fatty food samples for organochlorine pesticides by using a 20-g Florisil column and by eluting the pesticides with 200 mL of 6 percent ether in

TABLE A-21. COLUMN CHROMATOGRAPHIC CLEANUP OF ORGANOCHLORINE PESTICIDES AND PCBs USING FLORISIL

Column size (mm)	Adsorbent amount[a]	Deactivation	Sample	Solvent	Pesticide	Recovery (percent)	Ref.
--	1.6 g	Nil	Sediment extract	2:4:94 methanol:benzene:hexane 1:2:4:93 methanol:acetonitrile:benzene:hexane	Kepone	89 to 109	25
400 x 20	20 g	Nil	Food	6:94 ether:PE	Multi-OCS	40 to 100	69
400 x 20	100 mm	Nil	Food	6:94 ether:PE; 15:85 ether:PE	21 OCS	--	77
10 mm ID	10 g	2 percent H_2O	Milk	Hexane	PCBs	Varies with congener	73
600 x 40	100 g	5 percent H_2O	Butterfat	15 to 20 percent methylene chlorine in PE	Multi-OCS	100	78
400 x 20	20 g	Nil	Standards	6:94 ether:PE; 15:85 ether:PE	Multi-OCS	85 to 100	74
300 x 22	20 g	Nil	Butterfat Soybean oil	30 percent water in acetonitrile	Multi-OCS	>80	79
400 x 20	125 mm	5 percent H_2O	Butterfat	15:85 methylene chloride:PE	DDE, DDD, DDT, endrin, dieldrin	--	80
400 x 20	75 to 100 mm	0.7 percent H_2O	Standards	6:94 ether:PE; 15:85 ether:PE	Multi-OCS	91 to 100	81
500 x 15	30 g	Nil	Food	PE 5:1 benzene:PE; chloroform acetone	Multi-OCS	--	82
300 x 25	28 g	2 percent H_2O	Fish	Hexane; 30:70 methylene chloride:hexane	Multi-OCS	94 to 100	72
140 x 5	30 mm	Nil	Water	6:94 ether:hexane; 15:85 ether:hexane	Multi-OCS	96 to 100	83
300 x 22	20 g	Nil	Food	20:80 methylene chloride:hexane; 50:0.35:49.65 methylene chloride:acetonitrile:hexane; 50:1.5:48.5 methylene chloride:acetonitrile:hexane	Multi-OCS	>90 except endrin 60 to 90, heptachlor 80	84
400 x 20	100 mm	Nil	Fish	10:90 ether:PE	Multi-OCS	96 to 100	85
300 x 25	150 mm	2 percent H_2O	Food	Hexane; 5, 10, 15, 30 percent methylene chloride in hexane; 5, 10, 30 percent ether in hexane	Multi-OCS	--	86

(continued)

[a] The values given represent the amounts in grams or the heights of the adsorbed beds in the columns, as specified.

[b] Adjust weight of Florisil used according to the lauric acid adsorption value and ascertain that heptachlor epoxide, dieldrin, and endrin are being eluted by the appropriate eluant.

[c] $AgNO_3$-coated Florisil was used to separate phthalate esters from organochlorine pesticides.

OCS - organochlorine pesticides.

PE - petroleum ether.

TABLE A-21. (concluded)

Column size (mm)	Adsorbent amount[a]	Deactivation	Sample	Solvent	Pesticide	Recovery (percent)	Ref.
300 x 25	150 mm	10 percent H_2O	Food	Ether; Hexane; 5, 10, 15, 30 percent methylene chloride in hexane; 5, 10, 30 percent ether in hexane	Multi-OCS	--	86
300 x 10	5 g	Nil	Standards	6:94 ether:PE; 15:85 ether:PE 50:50 ether:PE	Multi-OCS	94 to 100	87
20 mm ID	100 mm	Nil	Water	Hexane	PCBs	--	68
--	b	Not specified	Food	20 percent methylene chloride in hexane (I); 50 percent methylene chloride; 0.35 percent acetonitrile; 49.6 percent hexane (II); 50 percent methylene chloride; 1.5 percent acetonitrile; 48.5 percent hexane (III)	Multi-OCS	--	88
15 mm ID	3 g	Nil[c]	Food	3 percent benzene in hexane	Multi-OCS	82 to 103	89
--	20 g	Nil	Sediment	35:65 ether:hexane; 5:10:85 benzene:methanol:hexane	Kepone	77 to 115	47

[a] The values given represent the amounts in grams or the heights of the adsorbed beds in the columns, as specified.

[b] Adjust weight of Florisil used according to the lauric acid adsorption value and ascertain that heptachlor epoxide, dieldrin, and endrin are being eluted by the appropriate eluant.

[c] $AgNO_3$-coated Florisil was used to separate phthalate esters from organochlorine pesticides.

OCS - organochlorine pesticides.

PE - petroleum ether.

petroleum ether. Later, Johnson (70) extended the cleanup procedure to dieldrin and endrin by eluting the Florisil with 15 percent ether in petroleum ether.

Burke and Mills (71) reported that endosulfan was recovered from the Florisil column by elution with 30 percent ether in petroleum ether.

To separate PCBs from pesticides, Reynolds and Copper (72) extended the Florisil procedure by introducing a hexane or a petroleum ether fraction to the 6 percent and 15 percent ether in petroleum ether.

Mills et al. (84) recommended an elution system which replaced the ether/petroleum ether mixture for multiple pesticide analysis. The three eluting mixtures are identified in Table A-22.

Difficulties in obtaining reproducible recoveries and the overlapping of pesticides between fractions have been reported (74, 75, and 76). This was attributed to the variation of Florisil among batches because of varying calcination temperatures and times.

TABLE A-22. MILLS 1972 MODIFIED PROCEDURE FOR ELUTION OF ORGANOCHLORINE PESTICIDES FROM FLORISIL[a]

Fraction I	Fraction II	Fraction III
20 percent methylene chloride in hexane (200 mL)	50 percent methylene chloride/0.35 percent acetonitrile/49.65 percent hexane (200 mL)	50 percent methylene chloride/1.5 percent acetonitrile/ 48.5 percent hexane
Aldrin	Dieldrin	
alpha-BHC	alpha-Endosulfan	
beta-BHC	beta-Endosulfan	
gamma-BHC	Endrin	
Chlordane	Heptachlor epoxide	
2,4'-DDE	Methoxychlor	
4,4'-DDE		
2,4'-DDT		
Heptachlor		
Hexachlorobenzene		
Mirex		
Perthane		
PCBs		
Toxaphene		

[a] Recoveries >90 percent, except for endrin (60 to 90 percent) and heptachlor (80 percent); data taken from Ref. 84.

Burke and Malone (74) studied the calcination conditions used in the manufacture of Florisil and reported that 1,250°F/3-hour calcination is the desirable condition for activation. They have found that as the calcination temperature is increased from 1,000 to 1,400°F, both pesticides and lipid materials are removed more readily from the column. Similar effects have been observed for increased calcination time (see Table A-23).

2.3.4.2 Silica Gel

Silica gel has been recommended for the separation of PCBs and pentachloronaphthalenes from organochlorine pesticides (90 and 91). Armour and Burke (90) reported separation of PCBs and organochlorine pesticides when a mixture of silica gel deactivated with 3 percent water and Celite 545 was used. Only aldrin could not be separated from the PCBs, but the other organochlorine pesticides, including lindane, heptachlor, heptachlor epoxide, dieldrin, endrin, DDT, DDE, and DDD, were recovered quantitatively with acetonitrile:hexane:methylene chloride (see Table A-24). With more highly deactivated silica gel, an overlap of organochlorine pesticides and PCBs was observed (Figure A-1).

Similar results were reported by Leoni (91) with silica gel deactivated with 5 percent water (Table A-25). The adsorbent in this case was silica gel grade 950, 60 to 200 mesh, Davidson code 950-08-08-226, Grace Davidson Chemical, Baltimore, Maryland. The silica gel was activated for 2 hours at 130°C and was then partially deactivated with distilled water (5 percent by weight). The compounds listed in Table A-25 were eluted from the silica gel column with hexane (20 mL), 60 percent benzene in hexane (8 mL), benzene (8 mL), and 50 percent ethyl acetate in benzene (14 mL).

Biddleman et al. (92) investigated silica gel deactivated with 3.3 percent water for the separation of PCBs, cis- and trans-chlordane, DDE, and DDT from toxaphene. The chlorinated hydrocarbons were eluted from the silica gel column in three fractions. Fraction I (50 mL petroleum ether) contained the PCBs, DDE, and 10 to 30 percent 2,4'-DDT. Fraction II (80 mL petroleum ether) contained chlordanes, 4,4'-DDT, and 70 to 90 percent 2,4'-DDT. Dieldrin and 4,4'-DDD were eluted in a third (15 mL dichloromethane) fraction. The percentages of toxaphene eluted in the three fractions were 10, 30, and 60 percent, respectively. Nonseparation of aldrin from PCBs is not inconvenient unless significant amounts of PCBs with a low degree of chlorination are present, since aldrin has a retention time that is different from those of PCBs with a higher chlorine content.

Acurex has slightly modified this fractionation technique to make it applicable to all of the organochlorine pesticides listed in Method 8080, including Kepone. The results are given in Table A-26. Other applications in which silica gel was used for the cleanup of organochlorine pesticides and PCB extracts are given in Table A-27.

TABLE A-23. PESTICIDE RECOVERIES AND FAT ELUTION FROM FLORISIL OF DIFFERENT CALCINATIONS

Calcination		Lindane[a]	Hepta-chlor[a]	Aldrin[a]	Heptachlor epoxide		4,4'-DDT[a]	Dieldrin		Endrin		Fat Residue (mg)	
Temp. (°F)	Time (hr)	6 percent eluate	6 percent eluate	6 percent eluate	6 percent eluate	15 percent eluate	6 percent eluate	6 percent eluate	15 percent eluate	6 percent eluate	15 percent eluate	6 percent eluate	15 percent eluate
1,000	2	99	37	96	0	90	94	0	0	0	0	0	15
1,100	2	99	18	95	0	91	89	0	0	0	0	0	48
1,200	2	101	11	98	0	92	97	0	0	0	0	0	102
1,200	4	97	43	96	95	2	100	0	95	0	98	0	213
1,200	8	97	99	101	99	0	108	23	67	64	21	35	275
1,200	16	101	102	102	103	0	98	0	99	8	88	43	239
1,250	2	98	94	100	100	0	106	0	101	0	102	1	224
1,250	4	97	41	97	16	86	103	0	92	0	98	0	215
1,250	8	101	92	101	101	0	106	0	101	0	102	1	272
1,250	16	100	96	102	98	0	117	0	97	0	100	0	244
1,300	2	99	38	96	95	2	100	0	81	0	79	0	224
1,300	4	99	81	100	100	0	95	0	96	2	98	31	267
1,300	8	101	99	102	99	0	97	0	100	0	100	0	213
1,300	16	101	100	103	100	0	102	40	42	79	7	127	203
1,350	2	97	95	97	97	0	100	60	25	90	3	25	291
1,350	4	102	92	102	100	0	102	0	101	12	78	136	181
1,350	8	99	102	93	99	0	100	100	0	100	0	289	65
1,350	16	101	101	103	100	0	99	99	0	99	0	266	80
1,400	2	98	95	99	100	0	98	30	55	75	13	86	199

[a] Lindane, heptachlor, aldrin, and 4,4'-DDT were not detected in any of the 15-percent eluates. Data taken from Ref. 74.

TABLE A-24. ELUTION PATTERNS OF ORGANOCHLORINE PESTICIDES AND PCBs USING ARMOUR/BURKE'S PROCEDURE WITH SILICA GEL/CELITE 545

Column packing	Fraction I 250 mL petroleum ether	Fraction II 200 mL acetonitrile:hexane: methylene chloride (1:19:80)
Silica gel (3 percent H_2O) plus Celite 545	PCB-1254 (97.5 percent)	Lindane (96 percent)
	PCB-1260 (97.5 percent)	Heptachlor (93 percent)
	Aldrin (101 percent)	Heptachlor epoxide (100 percent) Dieldrin (104 percent) Endrin (94 percent) 4,4'-DDE (95 percent) 2,4'-DDT (98 percent) 4,4'-DDT (98 percent) 4,4'-DDD (98 percent)

Note: Celite was mixed with the silicic acid to achieve a faster elution rate from the column. Silicic acid -- Mallinckrodt, 100 mesh powder, specially prepared for chromatographic analysis. Activate for 7 hours at 130°C; deactivate with 3 percent water; equilibrate for 15 hours. Celite 545 -- acid-washed (Johns-Manville).

2.3.4.3 Alumina

Alumina was used in the cleanup of extracts of organochlorine pesticides from animal fats and tissues (62), fish (97, 102), food (103, 104), vegetables (105), water (83, 106), and sediment (42). Table A-28 identifies several of the applications reported in the literature in which alumina was used for cleanup of the organochlorine pesticides and PCBs. Besides being an excellent adsorbent for fat, alumina has been found by Goerlitz and Law (42) to be more efficient than silica gel and Florisil in the removal of naturally occurring organic acids, pigments, and other interferences.

A multiple organochlorine pesticides analysis using alumina deactivated with 5 percent water for cleanup of fats was reported by de Faubert Mauder et al. (62). Lindane, dieldrin, DDT, DDE, and heptachlor epoxide were eluted from the column with hexane. Boyle et al. (102) used alumina deactivated with 1.0 to 1.5 percent water and fractionated 13 organochlorine pesticides by elution with 10 percent ether in hexane and collection of 50 mL-eluate fractions. Recoveries of various organochlorine pesticides ranged between 91

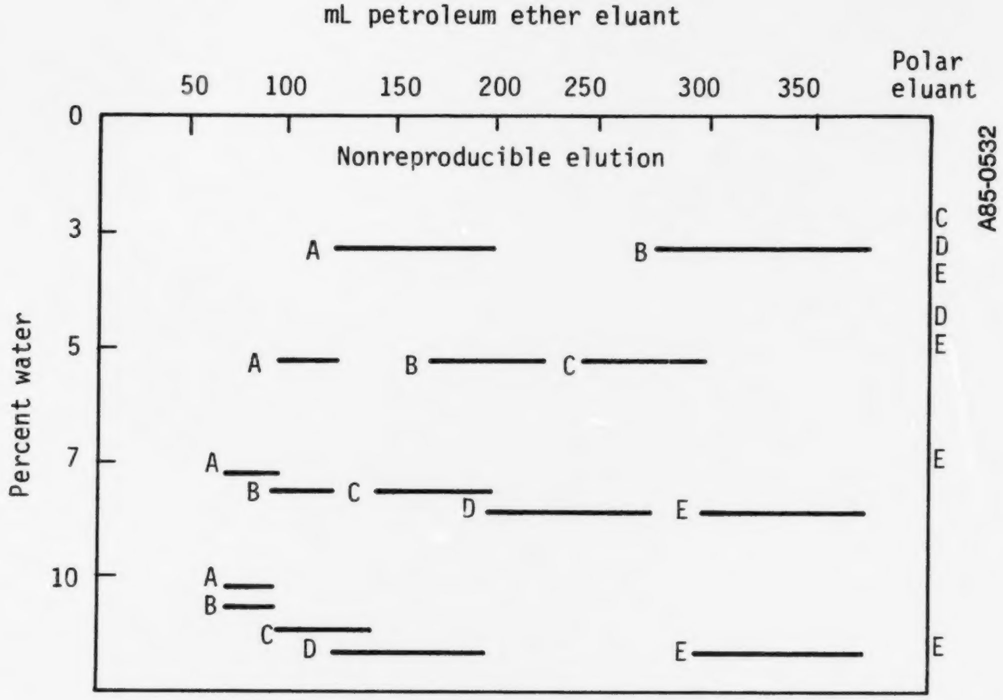


Figure A-1. Effect of silicic acid water content on elution and separation of PCB-1260 and DDT analogs. 25-mL fractions of petroleum ether eluate analyzed by GC; respective compound found present in volumes crossed by horizontal line. Various polar eluants used to eluate pesticides C, D, and E. A, PCB-1260; B, 4,4'-DDE; C, 4,4'-DDT; D, 2,4'-DDT; E, 4,4'-TDE.

TABLE A-25. ELUTION PATTERNS OF ORGANOCHLORINE PESTICIDES AND PCBs USING LEONI'S SILICA GEL PROCEDURE (DEACTIVATED WITH 5 PERCENT WATER)[a]

Compound	Hexane (20 mL)	60 percent benzene in hexane (8 mL)	Benzene (8 mL)
delta-BHC		+	
Heptachlor	+		
Aldrin	+		
Heptachlor epoxide		+	
Endosulfan I		+	
Dieldrin		+	
Endosulfan II		+	+
Endrin aldehyde (no data)			
DDT	+		
alpha-BHC		+	
beta-BHC		+	
gamma-BHC		+	
DDE	+		
Endrin		+	
DDD (no data)			
Endosulfan sulfate (no data)			
PCBs	+		
Chlordane	+	+	
Toxaphene (no data)			
Kepone (no data)			
Methoxychlor			+

[a] Data taken from Ref. 91.

and 100 percent except for lindane which was only 40 percent. The low recovery for lindane was attributed to the alkalinity of the alumina since on acidic and neutral alumina all recoveries were quantitative.

Telling (104) reported significant degradation of beta-BHC, alpha-endosulfan, and DDT on aluminas of activity higher than 2.5 on the Brockman scale. Partial separation of PCBs and organochlorine pesticides was reported on alumina of 2.5 activity (104).

Millar et al. (4) used the alumina procedure developed by Telling (104) to clean up wastewater extracts containing organochlorine pesticides and PCBs, and compared the results with those obtained using Florisil chromatography. The authors concluded that recoveries of spikes from both columns were excellent, and there was little reason to choose one

TABLE A-26. ELUTION PATTERNS OF ORGANOCHLORINE PESTICIDES AND PCBs USING BIDDLEMAN'S ET AL. SILICA GEL PROCEDURE (MODIFIED BY ACUREX)

Compound	Fraction I (50 mL hexane)	Fraction II (80 mL hexane)	Fraction III (15 mL methylene chloride)	Fraction IV (50 mL ethyl acetate)
gamma-BHC			+	
Heptachlor	+	+		
Aldrin	+	+		
Heptachlor epoxide			+	
Endosulfan I			+	
Dieldrin			+	
Endosulfan II			+	
Endrin aldehyde			+	+
4,4'-DDT		+	+	
alpha-BHC			+	
beta-BHC			+	
delta-BHC			+	
4,4'-DDE	+	+	+	
Endrin			+	
4,4'-DDD			+	
Endosulfan sulfate			+	
PCBs	+			
Chlordane	+	+	+	
Toxaphene	+	+	+	
Methoxychlor				
Kepone				+

Note:

With silicic acid (20 g) deactivated with 3 percent water/Celite (5 g), aldrin elutes in Fraction I (250 mL petroleum ether); heptachlor, DDE, DDT elute in Fraction II [200 mL acetonitrile:hexane:methylene chloride (1:19:80)] (90).

cleanup procedure over the other. However, they did observe that pesticides were much more frequently found in two fractions when an alumina column was used, and chlordane and toxaphene were often spread over three fractions.

2.3.5 High-Pressure Liquid Chromatography

The use of HPLC in the cleanup of environmental samples prior to analysis has significant potential primarily because of the possibility of

TABLE A-27. COLUMN CHROMATOGRAPHIC CLEANUP OF ORGANOCHLORINE PESTICIDES AND PCBs USING SILICA GEL

Column size (mm)	Adsorbent amount[a]	Percent deactivation (water)	Sample	Solvent	Pesticide	Recovery (percent)	Ref.
5 to 22 mm ID	1 to 20 g	5µL water per mL hexane	Soil, plant tissue, water	Hexane, mixtures of hexane and benzene, benzene	17 individual OCS	98 to 100	93 to 95
450 x 6	2 g	5	Fat, tissue	Hexane 10:90 ether:hexane	Multi-OCS	100	96
400 x 22	20 g silica gel + 5 g acid-washed Celite	3	Fish	PE 1:19:80 acetonitrile:hexane:methylene chloride	PCBs and DDT analogs	OCS = 80 to 107	90
140 x 5	30 mm	5	Water	Hexane 15:85 benzene:hexane 50:50 Benzene:hexane 20:80 ether:hexane	Multi-OCS	93 to 100	83
230 x 4		0.5 percent ether in benzene	Standards	Hexane 0.5 to 99.5 ether:benzene	PCBs and DDT analogs	--	97
6 mm ID	1 g	1	Water	10:90 benzene:hexane 60:40 benzene:hexane	Multi	--	98
450 x 7	2 g	3	Standards	Hexane 10:90 ether:hexane	PCBs and OCS	Average 92	99
300 x 4.2	1 g	5	Water	Hexane 60:40 benzene:hexane Benzene	PCBs and OCS	95 to 100	91
200 x 10	3 g	Nil	Fish	Pentane, benzene	PCBs and DDT analogs	OCS = 72 to 118	100
300 x 10	80 mm	3	Sediment	Hexane, benzene	PCBs and OCS	OCS = 75 to 99	42
300 x 10	5 g	"As is"	Standards	PE 1:19:80 acetonitrile:hexane:methylene chloride	PCBs and DDT analogs	OCS = 80 to 95	101

[a] The values given represent the amounts in grams or the heights of the adsorbent beds in the columns, as specified.
OCS - organochlorine pesticides.
PE - petroleum ether.

TABLE A-28. COLUMN CHROMATOGRAPHIC CLEANUP OF ORGANOCHLORINE PESTICIDES AND PCBs USING ALUMINA

Column size (mm)	Adsorbent amount[a]	Percent deactivation (water)	Sample	Solvent	Pesticide	Recovery (percent)	Ref.
--	10 g	5	Animal fats, tissue	Hexane	Multi-OCS	73 to 94	62
20 mm ID	150 mm	1 to 1.5	Fish	10:90 ether:hexane	Multi-OCS	91 to 100	102
200 x 6	2 g	15	Food	PE	Multi-OCS	40 to 90	103
300 x 10	8 g	15	Vegetable	Hexane 2:98 acetone:hexane	Multi-OCS	>90 except endosulfan 75	105
450 x 6	2 g	5	Fat, tissue corn oil	Hexane	Multi-OCS	97 to 109	96
140 x 5	30 mm	5	Water	Hexane 50:50 benzene:hexane	Multi-OCS	94 to 100	83
75 x 5	1.7 g	1 percent methanol in benzene	Fish, plankton	Hexane	OCS, PCBs	--	97
300 x 10	80 mm	9	Sediment	Hexane	OCS,PCBs	75 to 99	42
300 x 20	22 g	10	Food	Hexane	OCS and PCBs	70 to 124	104
450 x 6	3 g	4	Water, biota	Hexane	Multi-OCS	100	106

[a] The values given represent the amounts in grams or the heights of the adsorbent beds in the columns, as specified.

OCS -- organochlorine pesticides.

PE -- petroleum ether.

automation. Furthermore, in the case of water samples, the extraction and cleanup could be accomplished on the same setup. For example, a water sample can be first pumped through a reversed-phase Microbondapak-C_{18} column where the various organics including the organochlorine pesticides and PCBs are absorbed. The pesticides and PCBs are then eluted from the column by acetonitrile or another organic solvents, and appropriate fractions are collected for subsequent analysis (107).

Another application involving HPLC was reported by Larose (108). His system consisted of a liquid chromatograph (LC) equipped with a pellicular Corasil II column and a refractive index detector; petroleum ether was used as the mobile phase. Results reported by Larose indicated that fats, phospholipids, and oil were completely removed, and the recovery of lindane was better than 90 percent.

2.3.6 Selection of the Cleanup Technique

The choice of which adsorbent to use depends upon many factors, but the type of matrix and the interfering coextractants present seem to be the most important ones. Alumina has been found by Law and Goerlitz (83) to be more efficient than silica gel and Florisil in the removal of fats, naturally occurring organic acids, and pigments. Silica gel, on the other hand, was found to remove pigments that could not be removed with either alumina or Florisil.

When dealing with multicomponent analyses and especially in those cases where the GC analysis does not allow complete separation of all the organochlorine pesticides and PCBs, a combination of cleanup techniques may be required.

Goerlitz and Law (42) used Woelm neutral alumina, activity I (deactivated with 9 percent water), and silica gel deactivated with 3 percent water to separate PCBs and polychlorinated naphthalenes (PCNs) which interfere with the determination of the organochlorine pesticides (Figure A-2).

More polar compounds, such as phthalate esters, were separated from organochlorine pesticides and PCBs using alumina chromatography (109) as shown in Figure A-3.

In an attempt to improve the separation of organochlorine pesticides from PCBs, Berg et al. (110) used a combination of Florisil chromatography and activated charcoal chromatography (Figure A-4). The first fraction from the Florisil column was concentrated and applied to the charcoal column. Heptachlor and aldrin were eluted from charcoal with cyclohexane while the PCBs were recovered in the benzene fraction.

2.4 GC ANALYSIS

This review will address only the GC analysis with the electron capture detector (ECD) since this is the most widely used analytical technique in pesticide and PCB analysis. Basically, these compounds are separated by the chromatographic column, either packed or capillary, at elevated temperatures,

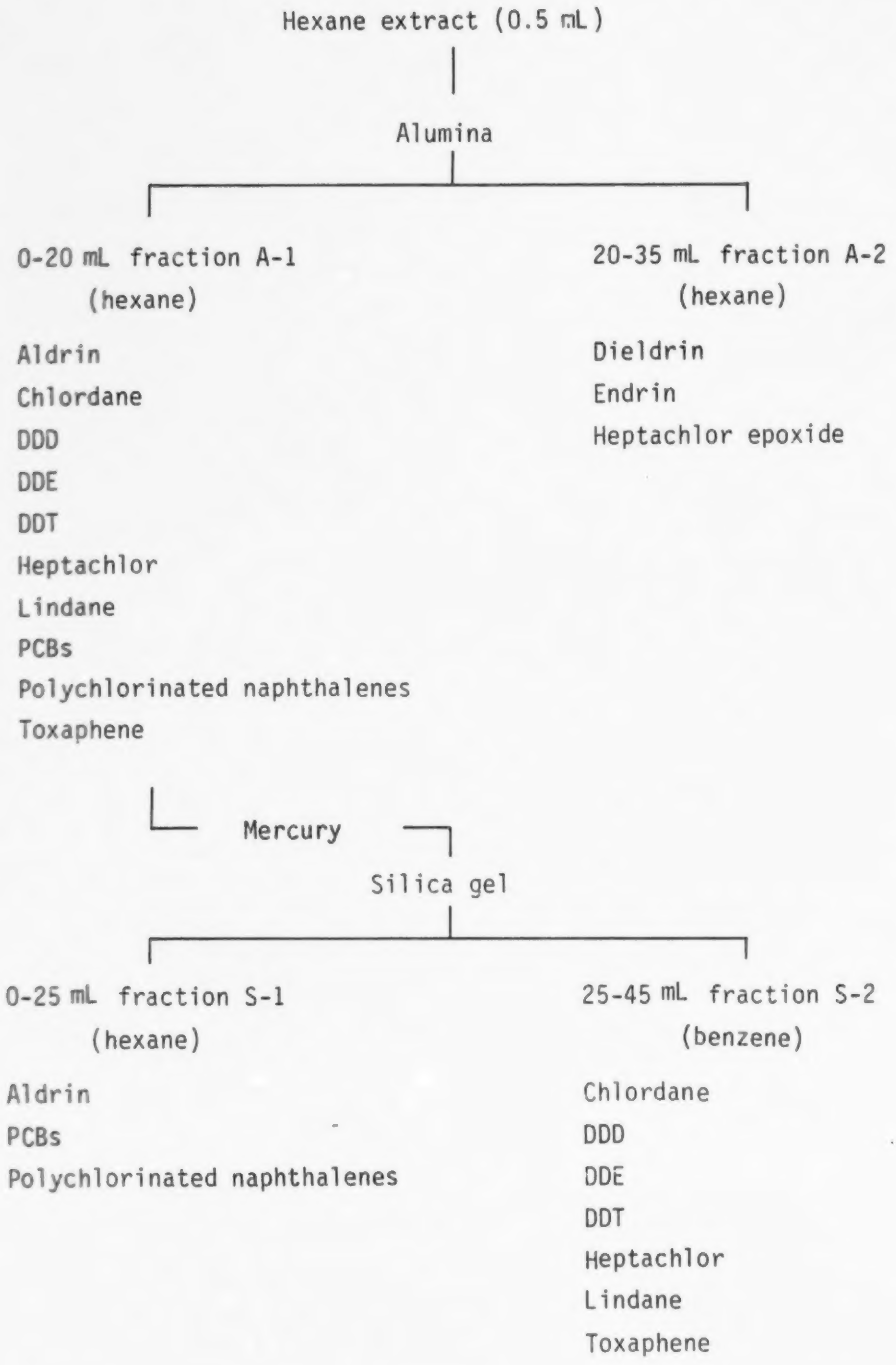
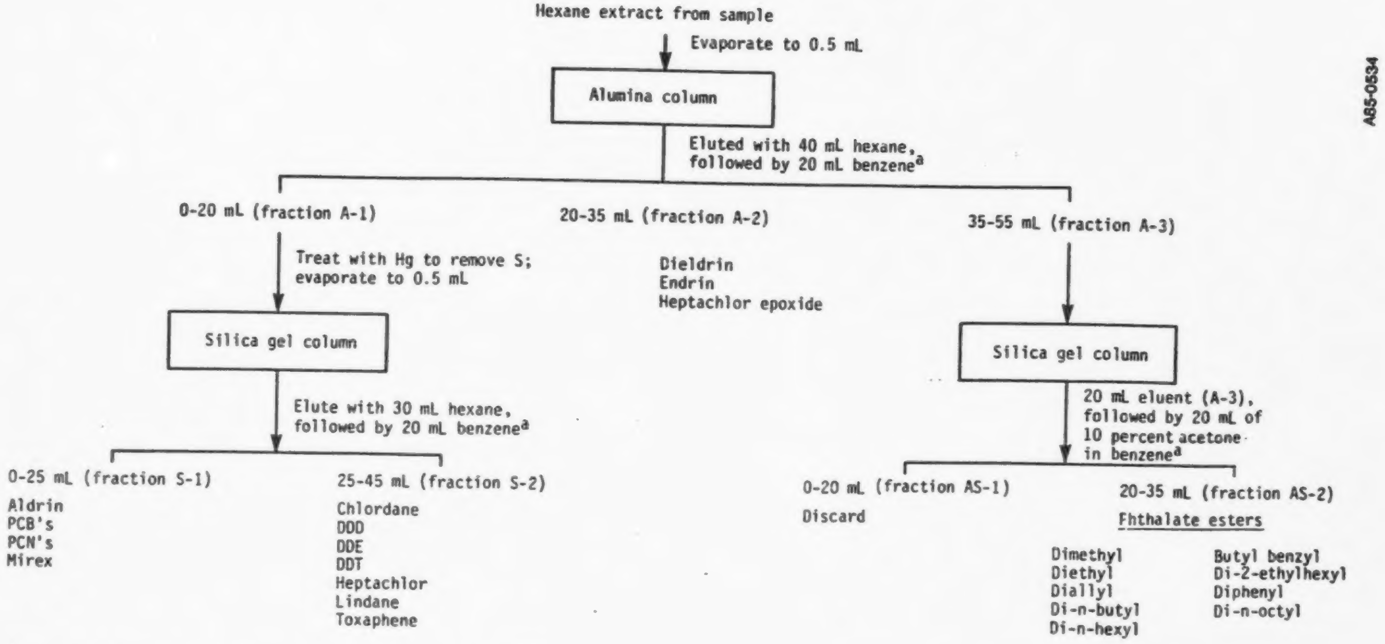

Hexane extract (0.5 mL)
Alumina
0-20 mL fraction A-1
(hexane)
Aldrin
Chlordane
DDD
DDE
DDT
Heptachlor
Lindane
PCBs
Polychlorinated naphthalenes
Toxaphene
20-35 mL fraction A-2
(hexane)
Dieldrin
Endrin
Heptachlor epoxide
Mercury
Silica gel
0-25 mL fraction S-1
(hexane)
Aldrin
PCBs
Polychlorinated naphthalenes
25-45 mL fraction S-2
(benzene)
Chlordane
DDD
DDE
DDT
Heptachlor
Lindane
Toxaphene

Figure A-2. Fractionation scheme employing Woelm neutral alumina, activity I, and silica gel deactivated with 3 percent water for the separation of organochlorine pesticides and PCBs. Data taken from Ref. 42.


Hexane extract from sample
Evaporate to 0.5 mL
Alumina column
Eluted with 40 mL hexane, followed by 20 mL benzene[a]
0-20 mL (fraction A-1)
20-35 mL (fraction A-2)
35-55 mL (fraction A-3)
Treat with Hg to remove S; evaporate to 0.5 mL
Dieldrin
Endrin
Heptachlor epoxide
Silica gel column
Silica gel column
Elute with 30 mL hexane, followed by 20 mL benzene[a]
20 mL eluent (A-3), followed by 20 mL of 10 percent acetone in benzene[a]
0-25 mL (fraction S-1)
25-45 mL (fraction S-2)
0-20 mL (fraction AS-1)
20-35 mL (fraction AS-2)
Aldrin
PCB's
PCN's
Mirex
Chlordane
DDD
DDE
DDT
Heptachlor
Lindane
Toxaphene
Discard
Fhthalate esters
Dimethyl
Diethyl
Diallyl
Di-n-butyl
Di-n-hexyl
Butyl benzyl
Di-2-ethylhexyl
Diphenyl
Di-n-octyl
A85-0534

[a] Approximately 5 ml of eluting liquid is retained by column

Figure A-3. Fractionation of organochlorine pesticides, PCBs, PCNs and phthalate esters using neutral alumina, activity I, and silica gel deactivated with 0.3 percent water (109).

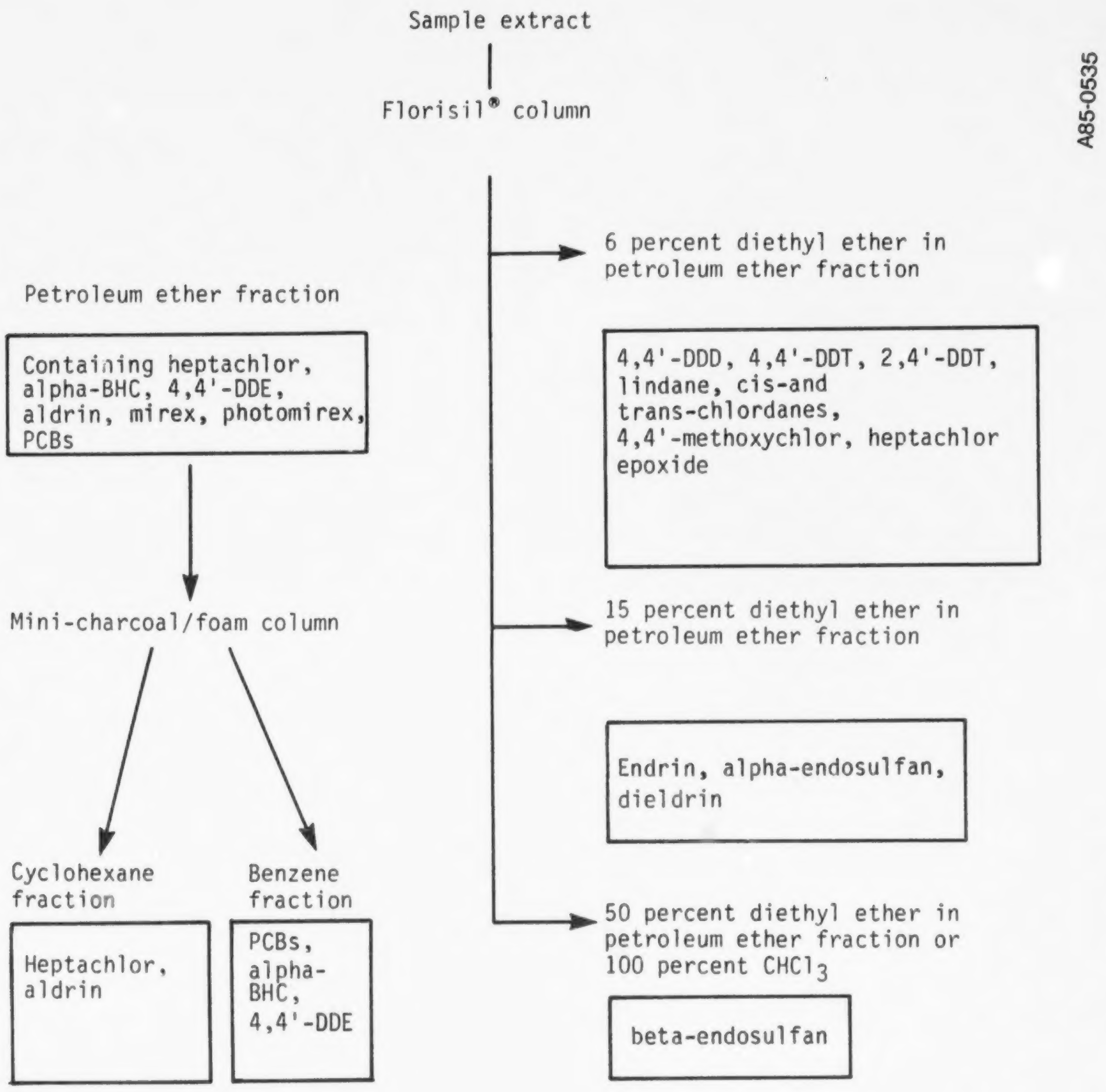

Sample extract
Florisil® column
6 percent diethyl ether in petroleum ether fraction
Petroleum ether fraction
Containing heptachlor, alpha-BHC, 4,4'-DDE, aldrin, mirex, photomirex, PCBs
4,4'-DDD, 4,4'-DDT, 2,4'-DDT, lindane, cis-and trans-chlordanes, 4,4'-methoxychlor, heptachlor epoxide
Mini-charcoal/foam column
15 percent diethyl ether in petroleum ether fraction
Endrin, alpha-endosulfan, dieldrin
Cyclohexane fraction
Benzene fraction
50 percent diethyl ether in petroleum ether fraction or 100 percent CHCl3
Heptachlor, aldrin
PCBs, alpha-BHC, 4,4'-DDE
beta-endosulfan
A85-0535

Figure A-4. Florisil/charcoal column fractionation of organochlorine pesticides and PCBs.

and the compounds are detected with an ECD. Other detectors, such as the microcoulometric or the modified Hall electrolytic conductivity detector, have also been used (1). Examples of organochlorine pesticide analysis by HPLC have been reviewed by Chau (1).

2.4.1 Gas Chromatographic Columns

Several factors should be considered when selecting columns for the GC determination of organochlorine pesticides and PCBs.

- The operating parameters should yield maximum resolution and detector response at minimal retention time.
- The stationary phase should be stable and not bleed during the GC analysis.
- The columns should stand up well under sustained periods of injection loading.

Columns of single and mixed stationary phases, both nonpolar and polar packed and capillary columns have been used in the analysis of organochlorine pesticides. Some of the more common ones are listed below. Table A-29 gives the relative retention times for several organochlorine pesticides.

- 1.5 percent OV-17 + 1.95 percent QF-1 (or OV-210) on Chromosorb W, HP, 100 to 120 mesh, 200°C and 60 mL/min carrier flowrate. By using this column, 4,4'-DDT is eluted in 20 min or less. This is an efficient column which completely resolves all common organochlorine pesticides except the partially resolved pairs beta-BHC and heptachlor, alpha-chlordane and alpha-endosulfan, 4,4'-DDD and beta-endosulfan, and 4,4'-DDE and dieldrin (Figure A-5).
- 4 percent SE-30 + 6 percent OV-210 (or QF-1) on Chromosorb W, HP, 80 to 100 mesh, 200°C, 70 mL/min flowrate. This column gives no separation of lindane and beta-BHC but does give good separation of 2,4'-DDT and 4,4'-DDD (Figure A-6).
- 4 percent OV-101 + 6 percent OV-210 on Chromosorb W, HP, 80 to 100 mesh, 200°C, 80 mL/min flowrate. This column has retention characteristics very similar to the above 4-percent SE-30 + 6-percent OV-210 (or QF-1) column and provides good separation of many organochlorine pesticides. Also, on-column decomposition of endrin is much reduced with this column when compared, for example, with the 4-percent DC-11 + 6-percent QF-1 column. Therefore, endrin and photoendrin can be resolved without excessive column preconditioning, and the detection limit for endrin is lowered. This column also gives sharp peaks and, thus, improved sensitivity for polar compounds such as 1-hydroxychlordene (decomposition product of heptachlor in water) and 1-hydroxy-3-chlorochlordene.
- 5 percent OV-210 (or QF-1) on Chromosorb W, HP, 100 to 120 mesh, 180°C, 60 mL/min flowrate. This packing gives complete separation of all common BHC isomers and fair separation among 4,4'-DDD, 4,4'-DDT, and heptachlor epoxide (Figure A-7).

TABLE A-29. RELATIVE RETENTION TIMES OF SOME COMMON ORGANOCHLORINE PESTICIDES

Pesticide	Column 1[a]	Column 2[b]	Column 3[c]	Column 4[d]	Column 5[e]	Column 6[f]	Column 7[g]	Column 8[h]	Column 9[i]
alpha-BHC	0.53	0.51	0.48	0.44	0.45	0.94	0.40	0.54	0.46
gamma-BHC	0.68	0.61	0.59	0.51	0.61	1.18	0.48	0.70	0.58
beta-BHC	0.78	0.62	0.60	0.48	0.74	--	0.43	0.84	0.59
Heptachlor	0.80	0.83	0.84	0.81	0.79	0.90	0.80	0.83	0.81
Aldrin	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Heptachlor epoxide	1.55	1.38	1.45	1.22	1.52	2.14	1.24	1.69	1.47
gamma-Chlordane	1.69	1.48	1.51	1.39	1.79	2.66	--	--	--
alpha-Chlordane	1.85	1.60	1.66	1.53	1.99	2.66	--	--	--
alpha-Endosulfan	1.94	1.70	1.80	1.51	1.98	2.47	1.56	2.14	1.88
4,4'-DDE	2.23	1.71	1.85	1.75	2.53	3.00	1.88	2.32	1.95
Dieldrin	2.38	2.00	2.15	1.75	2.46	3.34	1.84	2.65	2.24
Endrin	2.91	2.27	2.45	2.03	3.10	3.97	2.06	3.22	2.58
2,4'-DDT	3.16	2.20	2.40	2.32	3.77	4.24	2.54	3.23	2.58
4,4'-DDD	3.47	2.35	2.64	2.21	4.06	7.06	2.39	3.80	2.82
beta-Endosulfan	3.55	2.52	2.75	2.03	3.87	7.09	2.11	4.00	2.93
4,4'-DDT	4.17	2.83	3.20	2.86	4.95	7.07	3.18	4.46	3.37
Methoxychlor	8.20	3.96	4.58	4.21	10.38	14.81	4.93	8.5	5.30

[a] 1.8 m x 4 mm ID, 1.5 percent OV-17 + 1.95 percent OV-210, 200°C; data taken from Ref. 1.

[b] 1.8 m x 4 mm ID, 4 percent SE-30 + 6 percent OV-210, 200°C; data taken from Ref. 1.

[c] 1.8 m x 4 mm ID, 4 percent OV-101 + 6 percent OV-210, 200°C; data taken from Ref. 1.

[d] 1.8 m x 4 mm ID, 3 percent OV-101, 200°C; data taken from Ref. 1.

[e] 2.0 m x 4 mm ID, 3 percent OV-17, 198°C; data taken from Ref. 1.

[f] 1.8 m x 4 mm ID, 3 percent OV-225, 200°C; data taken from Ref. 1.

[g] 1.8 m x 4 mm ID, 10 percent DC-200 on Chromosorb W, HP, 80 to 100 mesh, 190°C; data taken from Ref. 27.

[h] 1.8 m x 4 mm ID, 1.6 percent OV-17 + 6.4 percent OV-210 on Chromosorb W, HP, 80 to 100 mesh, 190°C; data taken from Ref. 27.

[i] 1.8 m x 4 mm ID, 5 percent DC-200 + 7.5 percent OF-1 on Chromosorb W, HP, 80 to 100 mesh, 190°C; data taken from Ref. 27.

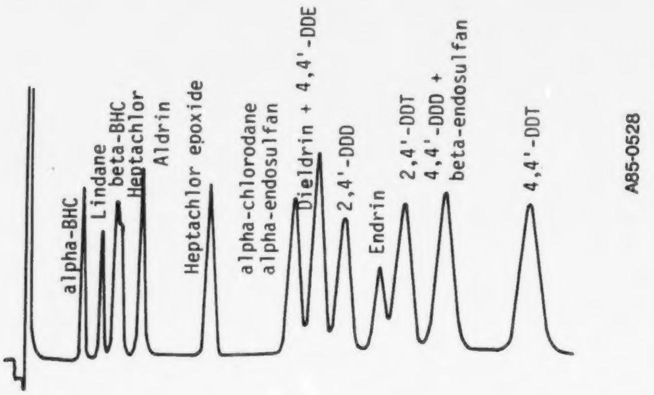


Figure A-5. 1.5 percent OV-17 + 1.95 percent OF-1.

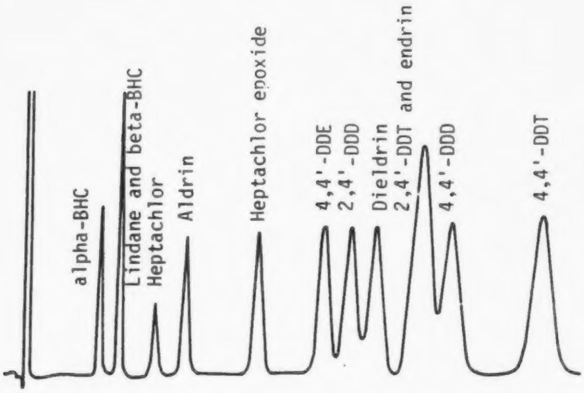


Figure A-6. 4 percent SE-30 + 6 percent OV-210.

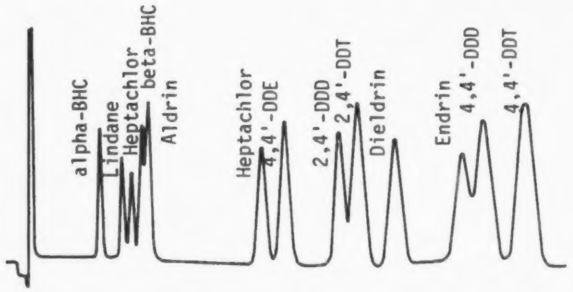


Figure A-7. 5 percent OV-210.

- 3 percent DEGS on Gas Chrom P, 80 to 100 mesh, 195°C, 70 mL/min flowrate. This gives excellent separation of BHC isomers as well as other commonly found organochlorine pesticides. The unusual elution pattern (beta-BHC after 2,4'-DDT, 4,4'-DDT before 4,4'-DDD) makes it useful for the confirmation of peaks (Figure A-8).

Other packing materials (10 percent DC-200; 1.6 percent OV-17 + 6.4 percent OV-210; 5 percent DC-200 + 7.5 percent QF-1) have been used for the organochlorine pesticides (Figures A-9 through A-11).

Resolution efficiencies for organochlorine pesticides and their related compounds with wall-coated open tubular columns (WCOT) and with support-coated open tubular (SCOT) glass capillary columns were compared with packed columns (111). The resolution index which is defined as the ratio of peak height to half-width of peak of standard injected increased in the order: packed $<<$ WCOT $\leq$ SCOT.

A GC/EC chromatogram of the organochlorine pesticides and PCBs obtained at Acurex on the fused-silica capillary column DB-5 (J&W Scientific, Rancho Cordova, CA) is shown in Figure A-12. Although not all the organochlorine pesticides were resolved from the mixture of PCB-1016 and -1260 on the fused-silica capillary column, the excellent resolution of the capillary column compared to the packed column (Figure A-13) resulted in the separation of over 50 components. Except for 4,4'-DDE and dieldrin, all compounds listed in Table A-30 have been resolved on the DB-5 fused-silica capillary column. Complete separation of 4,4'-DDE and dieldrin was achieved by Acurex on another fused-silica capillary column coated with SPB-608, and by Cooke and Ober (112) on an OV-17 + QF-1 capillary column.

2.4.2 Problems With Chromatography

2.4.2.1 Overloading

Symptoms of injection overloading include the gradual depression of the response, peak tailing, lowered column efficiency and resolution, and the on-column decomposition of certain compounds such as 4,4'-DDT. Figure A-14 provides an example of column overload with fatty materials (18 injections of 25 mg fat).

2.4.2.2 On-Column Decomposition of 4,4'-DDT and Endrin

Decomposition of 4,4'-DDT during the GC analysis is quite common and has been related to some problems in or adjacent to the front end of the column (113). Data from 19 laboratories performing organochlorine pesticide analyses are summarized in Table A-31. Severe decomposition of 4,4'-DDT in Laboratory 8 was related to the fact that the injection insert tube was not changed for 3 weeks prior to analysis.

Endrin decomposition has been reported to occur (Table A-32) whenever analysis was performed on a freshly prepared column (113). Silylation was reported to help in reducing endrin decomposition (Figure A-15).

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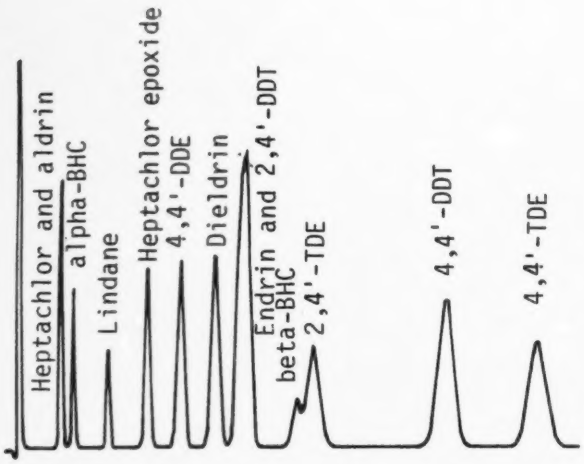

Figure A-8. 3 percent DEGS.

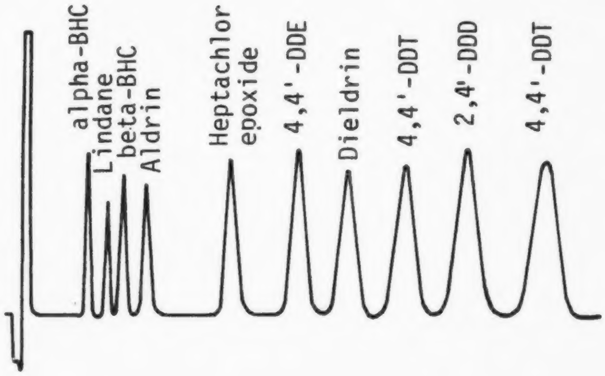

Figure A-9. 10 percent DC-200.

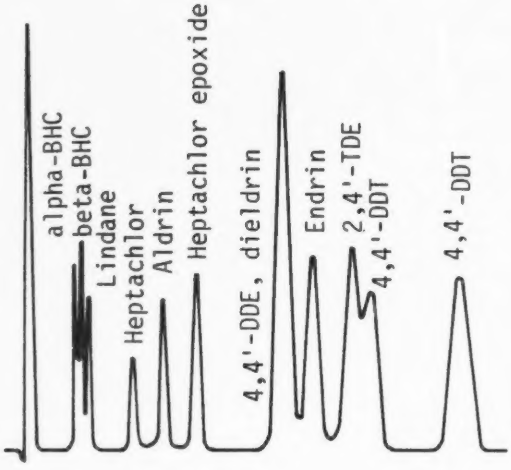

Figure A-10. 1.6 percent OF-17 +
6.4 percent OV-210.

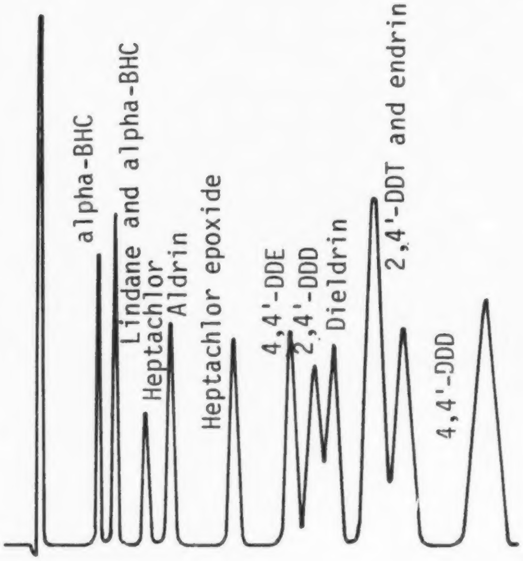

Figure 11. 5 percent DC-200 +
7.5 percent OF-1.

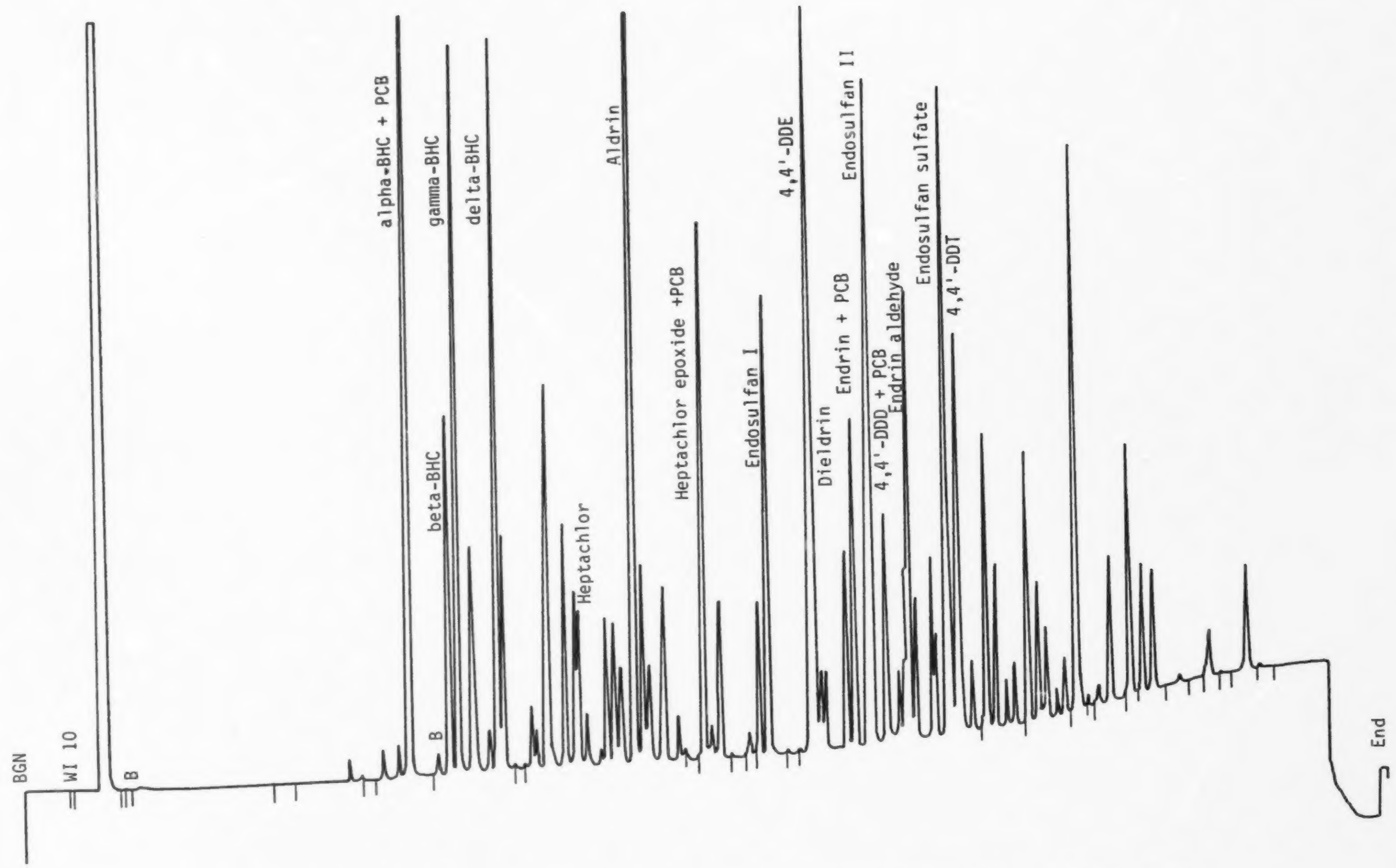

Figure A-12. GC/EC chromatogram of composite organochlorine pesticide and PCB-1016/1260 standard (30 m x 0.25 mm ID DB-5 fused-silica capillary column; 100 to 200 pg pesticides and 1,000 pg PCBs injected).

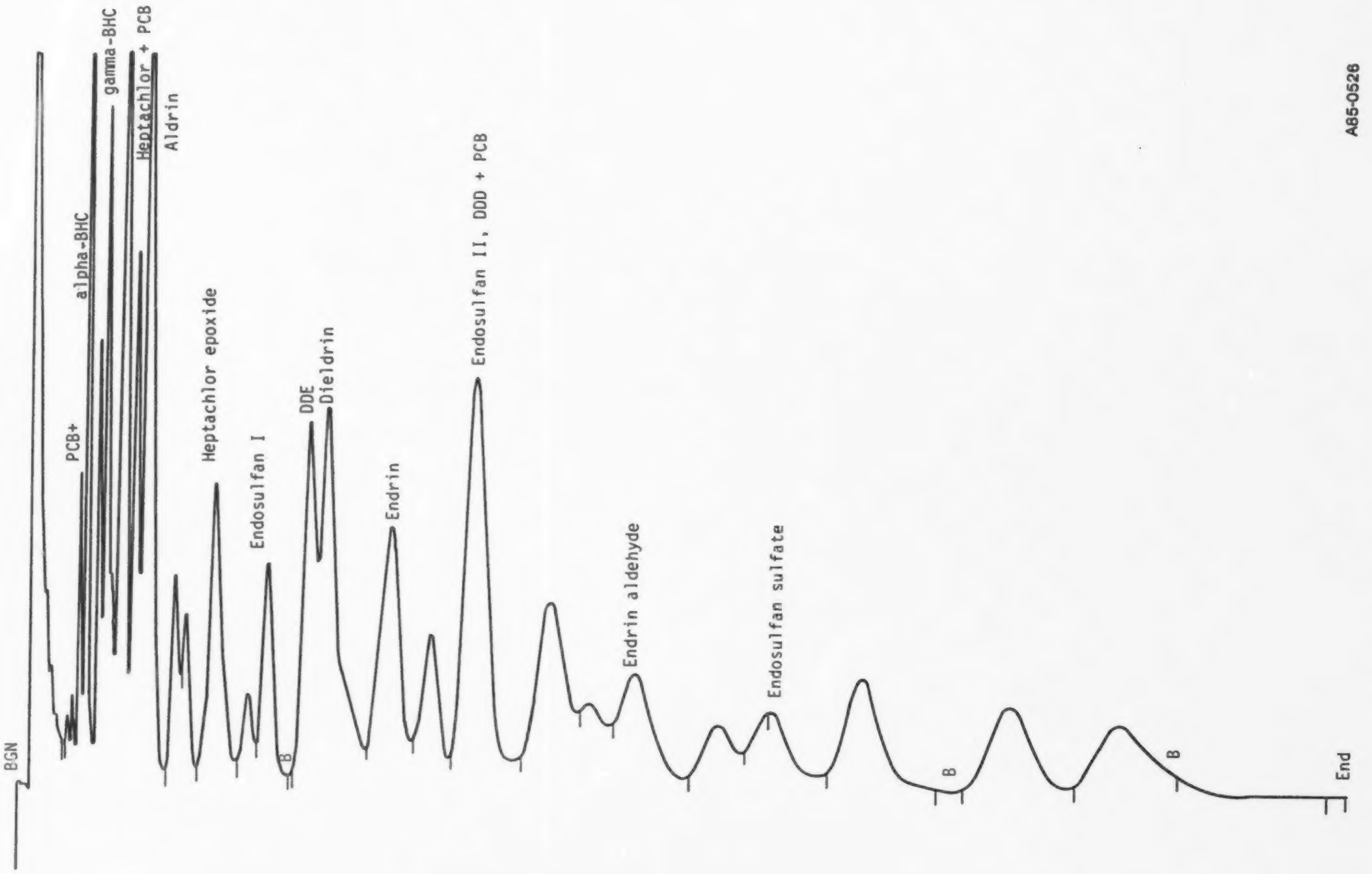

Figure A-13. GC/EC chromatogram of composite organochlorine pesticide and PCB-1016/1260 standard (1.8 m x 4mm ID packed 1.5 percent OV-17 + 1.95 percent OV-210; 25 to 50 pg pesticides and 500 pg PCBs injected).

TABLE A-30. SUMMARY OF PESTICIDE RETENTION TIMES (MIN) ON TWO FUSED-SILICA CAPILLARY COLUMNS[a]

Compound	DB-5[b]	SPB-608[c]
alpha-BHC	6.52	9.46
beta-BHC	7.28	11.33
gamma-BHC	7.49	10.97
delta-BHC	8.19	12.73
Heptachlor	9.83	12.46
Aldrin	11.02	13.76
Heptachlor epoxide	12.43	15.98
Endosulfan I	13.72	17.40
4,4'-DDE	14.68	18.36
Dieldrin	14.68	18.60
Kepone	16.72	D
Endrin	15.46	19.96
4,4'-DDD	15.80	20.53
Endosulfan II	16.20	20.69
4,4'-DDT	17.59	21.72
Endrin aldehyde	16.51	21.90
Endosulfan sulfate	17.39	22.54
Methoxychlor	19.77	24.90
gamma-Chlordane	13.26	16.70
alpha-Chlordane	13.82	17.31

D = degraded on-column.

[a] Data obtained at Acurex.

[b] Temperature program: 160°C (hold 2 min) to 270°C (hold 1 min) at 5°C/min.

[c] Temperature program: 160°C (hold 2 min) to 290°C (hold 1 min) at 5°C/min; nitrogen at 20 psi.

2.5 CONFIRMATION OF COMPOUND IDENTITY

Several techniques are available for the confirmation of compounds detected with the GC detectors. These include the multi-column confirmation technique, chemical derivatization followed by reanalysis of the extract, confirmation by gas chromatography/mass spectrometry (GC/MS), and fractionation of the extract followed by analysis.

Although the multi-column technique has been, and still is, a widely practiced procedure, there have been reports which claim that misinterpretation of pesticide identity occurred when the retention time was the only criterion

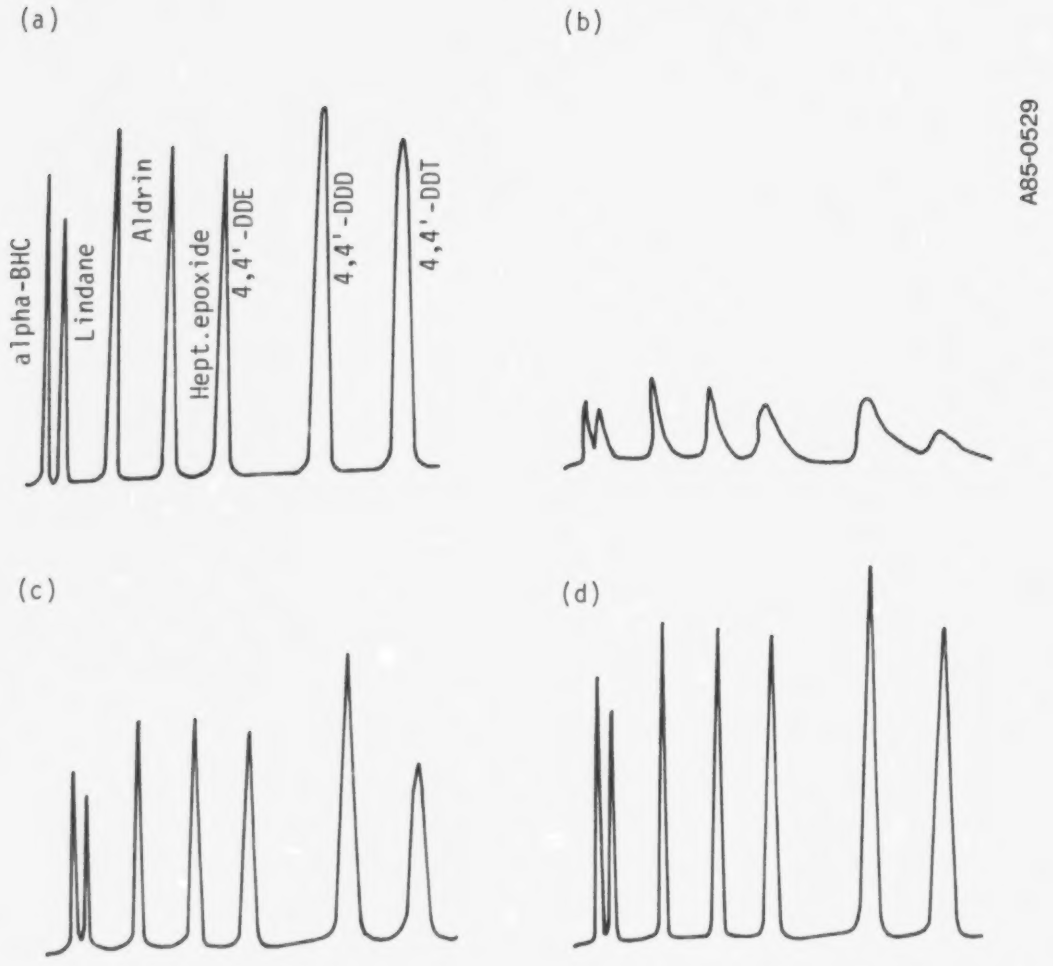

Figure A-14. Chromatograms illustrating column overloading and subsequent rejuvenation:

a. Freshly prepared column.

b. After 18 consecutive injections of 25 mg fat.

c. After 18 consecutive injections of fat, a clean glass insert was installed in the injection port, and the system was requilibrated for 30 min.

d. Complete rejuvenation of the system after overnight conditioning.

TABLE A-31. ON-COLUMN DECOMPOSITION OF 4,4-DDT[d]

OV-17 + QF-1					SE-30 + QF-1				
Lab. No.	Column age (days)	Est. total No. of injections	Glass insert changed (days)	Total decomposition (percent)	Lab No.	Column age (days)	Est. total No. of injections	Glass insert changed (days)	Total decomposition (percent)
14	21	0	21	None	5	126	500	1	None
17	12	0	1	None	17	13	75	1	None
12	49	e	1	None	15	142	1,300	1	None
1	47	20	6	None	12	49	e	1	None
4	257	800	10	Neglig.	6	70	125	2	None
15	144	1,300	1	Neglig.	14	46	150	1	Neglig.
19	38	146	1	Neglig.	13	186	1,500	1	Neglig.
5	70	250	1	1.4	7	73	60	5	Neglig.
13	186	1,500	1	2.2	16	e	3,000	5	3.2[a]
9	100	300	1	3.1	4	254	800	10	6.0
3	34	---	1	3.1	2	71	400	5	6.0
6	70	125	2	3.4	10	254	460	22	7.0
16	545	3,000	5	3.7[a]	9	110	150	1	8.6[a]
10	253	320	21	8.5[a]	18	4	None	--[b]	11.0[a]
2	71	400	5	14.0	11	5	30	5	13.0[a]
7	108	400	5	14.0	1	57	20	0	22.0[a]
18	4	45	--[b]	15.0[a]	8	141	200	23	25.0[a]
8	141	200	23	--[c]	3	35	e	1	--[c]

[a] Breakdown to 4,4'-DDD and 4,4'-DDE.

[b] Using on-column injection.

[c] Could not assess because of noise in baseline.

[d] Data taken from Ref. 113.

[e] Unknown.

TABLE A-32. ON-COLUMN DECOMPOSITION OF ENDRIN[a]

OV-17 + QF-1				SE-30 + QF-1			
Lab. No.	Est. total No. of injections	Column silylation	Total decomposition (percent)	Lab No.	Est. total No. of injections	Column silylation	Total decomposition (percent)
19	146	No	Neglig.	6	125	No	None
12	d	No	Neglig.	7	60	No	Neglig.
7	400	No	Neglig.	15	1,300	Yes	3.6
13	1,500	Yes	6	9	150	Yes	4.4
15	1,300	Yes	6	13	1,500	Yes	6.0
14	0	No	7.5	10	460	No	7.0
9	300	Yes	7.7	2	400	No	7.5
16	3,000	No	8	4	800	No	10
2	400	No	9	5	500	No	11
8	200	d	9	12	d	No	11
4	800	No	15	16	3,000	No	11
10	320	No	15	14	150	No	12
5	250	Yes	17	8	200	d	14
3	---	No	18	11	30	No	15
1	20	No	51	1	20	Yes	26
17	0	No	55	18	0	No	--b
11	30	No	--b	17	75	No	--b
18	45	No	--b	3	d	No	--b
6	125	No	--c				

[a] Data taken from Ref. 113.

[b] Did not allow chromatogram to run for full retention of last breakdown peak.

[c] Breakdown peaks obscured by noisy baseline.

[d] Unknown.

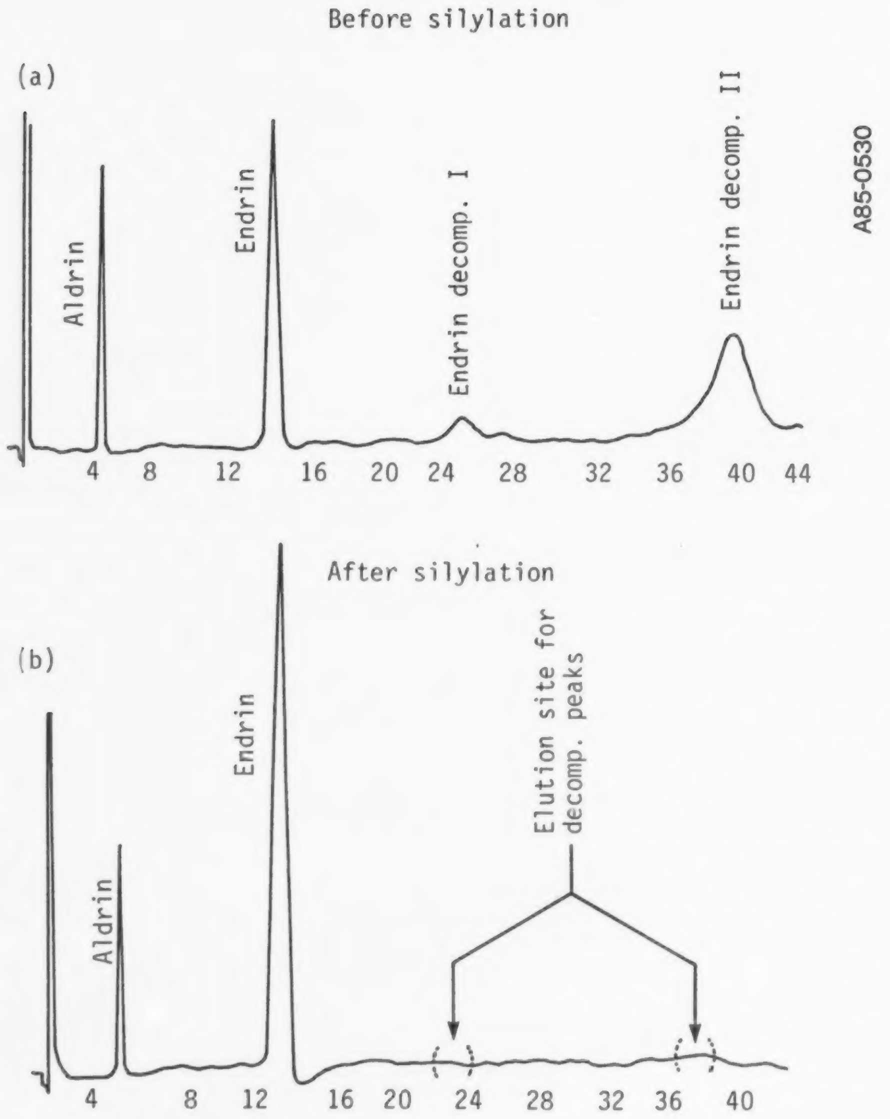

Figure A-15. Reduction in decomposition of endrin resulting from column silylation.

used in the identification. Therefore, two and three columns have been required to confirm the compounds when GC is used for analysis.

Chemical derivatization techniques have been used intensively for confirmation in pesticide residue work and will be addressed only briefly here since it is really beyond the scope of this project.

Confirmation of pesticide identity by GC/MS has been reported; this technique can only be applied to those samples in which the pesticide concentration is above approximately 5 µg/L for water and 50 ng/g for soil samples.

Fractionation of extracts using silica gel, alumina, Florisil, etc., has been addressed in Section 4.3.

Chemical Derivatization

A summary of the type of reactions used for the confirmation of organochlorine pesticides by chemical derivatization-GC techniques is presented in Table A-33. Applications of alkali dehydrochlorination for DDT and analogs are given in Table A-34, and summaries for chemical derivatization procedures for endrin/dieldrin, aldrin, the chlordane group, BHCs, and endosulfans are given in Tables A-35 through A-39.

In selecting the most appropriate method(s) for routine work, consideration should be given to those procedures that allow unambiguous confirmation of pesticide identity and that circumvent interference(s) in the quantitative analysis. Furthermore, the method should use routine instrumentation, should be easy to standardize, and should not include fractionation (the fractionation of an extract is not desirable since it increases the number of analyses).

2.6 STABILITY OF PESTICIDE SOLUTIONS

A study intended to identify the magnitude of solvent evaporation under normal laboratory conditions was reported by Hodgson (148 and 149). The factors studied include choice of solvent, type of container and closure, solution volume, and storage temperature. Long-term chemical stability of dilute standards was assessed under four storage conditions: freezer at -15°C; refrigerator at 3°C; ambient temperature in the dark, and ambient temperature on the bench top exposed to fluorescent and natural light. The conclusions of this study are summarized below.

- The evaporation rates of solvents from 10-mL glass-stopper volumetric flasks at ambient temperatures showed a direct relationship with the boiling point temperatures and with the ratios of vapor pressures to surface tensions. Table A-40 lists the experimental values for the evaporation rate and the ratios of vapor pressures to surface tensions. Isooctane and toluene are recommended as solvents for the organochlorine pesticides.

TABLE A-33. TYPE OF REACTIONS USED FOR THE CONFIRMATION OF ORGANOCHLORINE PESTICIDES BY CHEMICAL DERIVATIZATION -- GC TECHNIQUES

Type of reaction	Reagent	Pesticides
Dehydrochlorination to olefins	KOH or NaOH	DDT and analogs
	t-BuOK	DDT, analogs, and chlordanes
Reductive dechlorination	$CrCl_2$	Heptachlor + endrin
Addition	Bromination	Aldrin
	Chlorination	Aldrin
	t-BuOCl/t-BuOH	Aldrin + heptachlor
	t-BuOCl/AcOH	Aldrin + heptachlor
	Peracids	Aldrin + chlordene
Epoxide rearrangement	H_2SO_4	Endrin + dieldrin
	H_2SO_4/Ac_2O	Endrin + dieldrin
	BCl_3/2-Chloroethanol	Endrin + dieldrin
	$HCl/ZnCl_2$	Endrin + dieldrin
Oxidation	CrO_3	DDE + endrin + heptachlor
Intramolecular Rx (base-catalyzed)	KOH/ROH t-BuOK	alpha- + beta-endosulfans, Endrin cyclic ketones (degradation products of endrin)
Intramolecular Rx (acid-catalyzed)	H_2SO_4/Al_2O_3 (on SM reaction)	Endosulfans
	H_2SO_4 in solution H_2SO_4/Al_2O_3 (SM reaction)	Endrin
Cleavage of sulfite moiety	$LiAlH_4$ followed by silylation or acetylation	Endosulfans
	Ac_2O/H_2SO_4 (in solution)	Endosulfans
	$Ac_2O/H_2SO_4/Al_2O_3$ (SM reaction)	Endosulfans

TABLE A-34. APPLICATIONS OF ALKALI DEHYDROCHLORINATION FOR DDT AND ANALOGS

Compounds	Reagents	Reaction conditions	Known Interference	Reference
4,4-'DDT	2 percent NaOH in ethanol (10 mL)	Reflux, 20 min	Chlordanes	114
4,4'-DDT, 2,4'-DDT, 4,4'-DDD, perthane	2 percent NaOH in ethanol (10 mL)	Reflux, 20 min	Chlordanes	115
4,4'-DDT and DDD, 2,4'-DDT	0.5 N alcoholic KOH, (0.5 mL)	Room temperature, 5 min	Chlordanes	103
4,4'-DDD and DDT, methoxychlor	K_2CO_3 solid	Flash heater (240°C), RxGLC	Chlordanes	116
4,4'-DDT + DDD, 2,4'-DDT	2 percent KOH in 95 percent ethanol 5 mL	77°C, 15 min	Chlordanes	82
4,4'-and 2,4'-DDT	5 percent NaOH in ? mL	Reflux, 30 min	Chlordanes	53
4,4'-DDT, 2,4'-DDT, 4,4'-DDD, methoxychlor	Anhydrous NaOMe	Shake with 1 mL sample extract in hexane (1 min or 20 min) at room temperature	Chlordanes	117
4,4'-DDT, 2,4'-DDT, 4,4'-DDD, methoxychlor, perthane	NaOH + KOH on Gas Chrom Q	Alkaline pre-column (200°C, ?) RxGLC	Chlordanes	118
4,4'-DDT, 2,4'-DDT, 4,4'-DDD, methoxychlor	2 percent KOH in 95 percent ethanol	80°C, 12 min	Chlordanes	119
4,4'-DDT and DDD, chlordanes	t-BuOK/t-BuOH 200 mg/ℓ mL	Reflux (82 to 85°C) 30 min	--	120,121
2,4'- and 4,4'-DDT	NaOH, pH 8	30°C, 10 min	Chlordanes	122
2,4'-DDT; 2,4'-DDD perthane, methoxychlor	t-BuOK/t-BuOH 200 mg/2 mL	50°C or reflux 30 min	--	123
4,4'- and 2,4'-DDT, DDD, perthane, methoxychlor (and other pesticides)	2 percent KOH in 95 percent ethanol 2mL	Reflux at 100°C 15 min	Chlordanes	124
4,4'- and 2,4'-DDT, DDD perthane, methoxychlor	2 percent KOH in ethanol (1 mL)	Reflux and heat (15 min) to 0.2 mL	Chlordanes	125
DDT, DDD isomers, perthane, methoxychlor, chlordanes	Al_2O_3/KOH 200 g/20 g	Room temperature or 75°C, 1 hr and 3 hr	--	126
	Al_2O_3/t-BuOK 100 g/20 g	75°C, 1 hr	Chlordanes	126

TABLE A-35. SUMMARY OF CHEMICAL CONFIRMATION PROCEDURES FOR ENDRIN AND DIELDRIN

Compounds	Reagents	Reaction conditions	Known Interference	Reference
Endrin, dieldrin	0.5 mL (1:2, HBr Ac_2O)	room temperature, 30 min	--	103
Endrin	4 mL (15 g CrO_3 in 50 mL HOAc)	77°C, 12 min	--	82
Endrin, dieldrin	0.5 mL (0.1 g $ZnCl_2$ 2 mL HCl, 25 mL abs. EtOH)	100°C, 10 min	--	127
Endrin	0.5 mL H_2SO_4	room temperature, 10 to 15 min	Dieldrin in some GC column	121
Endrin, dieldrin	H_2SO_4/HOAc	100°C, 30 min	--	128
Endrin (heptachlor)	Acidic $CrCl_2$ sol. in acetone	55 to 60°C, 45 min	--	129
Endrin	$Al_2O_3/H_2SO_4/H_2O$ (100 g/10 mL/5 mL)	room temperature, 1-1/2 hr	--	130,131
Endrin (heptachlor)	$Al_2O_3/H_2SO_4/H_2O$ (50 g/10 mL/5 mL)	95 to 100°C, 1-1/2 hr	--	132
Two endrin photodegradation products	t-BuOK/t-BuOH followed by silylation or acetylation	65 to 70°C, 1-1/2 hr	--	133
Endrin dieldrin	BCl_3/2-ClEtOH	90°C, 2 hr 90°C, 10 min (endrin only)	--	134
Endrin, dieldrin (endosulfans, heptachlor epoxide)	HCl/Ac_2O (20 mL/10 mL)	room temperature, 30 min	--	135

TABLE A-36. SUMMARY OF CHEMICAL CONFIRMATION PROCEDURES FOR ALDRIN

Compounds	Reagents	Reaction conditions	Known Interference	Reference
Aldrin	$Cl_2/CHCl_3$	Room temperature, 5 min	--	103
Aldrin, (heptachlor, DDE)	CrO_3/HOAc	77°C, 12 min	Dieldrin	82
Aldrin	Peracetic acid	Reflux, 3 hr	Dieldrin	136
Aldrin	m-chloroperbenzoic acid/ hexane	43 to 45°C, 1-1/2 hr	Dieldrin	137,138
Aldrin	Monoterephthalic acid/ hexane	Room temperature, 20 min	Dieldrin	120
Aldrin, (heptachlor)	t-BuOCl/HOAc	100°C, 10 min	--	121
Aldrin	Chloroacetic anhydride/ HBr	56 to 60°C, 2 hr	Not investigated	139

TABLE A-37. SUMMARY OF CHEMICAL CONFIRMATION PROCEDURES FOR THE CHLORDANE GROUP

Compounds	Reagents	Reaction conditions	Known Interference	Reference
Heptachlor epoxide	0.5 mL HBr/Ac_2O	120°C, 30 min or room temperature, 30 min	--	103
Heptachlor, (Aldrin, DDE)	CrO_3/HOAc	77°C, 12 min	--	82
Heptachlor	AgOAc/HOAc	Reflux, 30 min	--	140
Heptachlor	Ag_2CO_3/50 percent aq. EtOH, then silylation or acetylation	Reflux, 45 min	--	140
Heptachlor, heptachlor epoxide, cis- and trans-chlordane (DDT + DDD isomers)	t-BuOK/t-BuOH, then silylation or acetylation	Reflux, 30 min	2- and 3-chlorochlordene, Nonachlor	120,121
Heptachlor (and endrin)	$CrCl_2$ aq. acidic sd.	55 to 60°C, 45 min	Chlordene	129
Heptachlor, heptachlor epoxide, cis- and trans-chlordane (DDT + DDD isomers, perthane, methoxychlor)	Al_2O_3/t-BuOK Al_2O_3/KOH	70 to 75°C, 1 hr or room temperature overnight	2- and 3-chlorochlordene	126
Heptachlor, endosulfans, endrin	Al_2O_3/H_2SO_4 (50 g/10 mL)	100°C, 1-1/2 hr	--	141
Heptachlor, endrin	$Al_2O_3/H_2SO_4/H_2O$ (50 g/10 mL/5 mL)	95 to 100°C, 1-1/2 hr	--	132
Heptachlor, heptachlor epoxide, endrin, dieldrin, endosulfans	0.5 mL HCl/Ac_2O	Room temperature, 30 min	Not fully investigated	135
Heptachlor, heptachlor epoxide	UV in petroleum ether (traces of MeOH)	30 min	Heptachlor + octachlor epoxide derivatives on some column; UV derivative of heptachlor epoxide and parent epoxide separable on OV-101 column	142

TABLE A-38. CHEMICAL CONFIRMATION OF BHC ISOMERS

Compounds	Reagents	Reaction conditions	Known Interference	Reference
BHC isomers (all)	2 percent KOH in ethanol	100°C, 15 min, GC column: 200°C	BHC isomers mutually interfere	124,125
BHC isomers (alpha, beta, gamma)	0.1 g NaOMe in MeOH (2 mL)	50°C, 15 min, GC column: 200°C	BHC isomers mutually interfere	143

TABLE A-39. SUMMARY OF CHEMICAL CONFIRMATION PROCEDURES FOR ENDOSULFANS

Compounds	Reagents	Reaction conditions	Known Interference	Reference
alpha- and beta-endosulfan	LiAlH$_4$/THF, then silylation or acetylation	60°C, 1 hr	--	144
	Ac$_2$O/HOAc/trace of H$_2$SO$_4$	100°C, 3/4 hr	--	144
	2 percent KOH in EtOH	Reflux, 12 min	Not investigated	145
	Al$_2$O$_3$/Ac$_2$O/H$_2$SO$_4$ (50 g/10 mL/5 mL)	100 ±5°C, 2 hr	--	146
	AL$_2$O$_3$/H$_2$SO$_4$ (50 g/10 mL)	95°C, 1 hr	--	147
alpha- and beta-endosulfan, (heptachlor)	AL$_2$O$_3$/H$_2$SO$_4$ (50 g/10 mL)	100°C, 1-1/2 hr	--	141
alpha- and beta-endosulfan (heptachlor epoxide, endrin, dieldrin)	0.5 mL HCl/Ac$_2$O	Room temperature 30 min	Not investigated	135

TABLE A-40. EVAPORATION RATES FROM 10-mL GLASS-STOPPER VOLUMETRIC FLASKS AND VAPOR PRESSURE/SURFACE TENSION RATIOS FOR SOME COMMON ORGANIC SOLVENTS[a]

Solvent	Vapor pressure/ surface tension ratio, torr (cm/dyne)	Evaporation rate (mL/wk ± SD)
Pentane	33.1	--
Ethyl ether	32.4	0.63 ± 0.17
Methylene chloride	16.0	0.25 ± 0.10
Hexane	8.45	0.15 ± 0.07
Acetone	8.01	0.22 ± 0.01
Chloroform	7.32	--
Methanol	5.65	0.076 ± 0.17
Ethyl acetate	3.97	--
Acetonitrile	3.79	--
Benzene	3.38	0.096 ± 0.0013
Ethanol	2.73	--
Isooctane	2.68	0.058 ± 0.022
Heptane	2.31	--
Isopropanol	2.11	--
Toluene	1.02	0.045 ± 0.0021
n-Decane	0.04	--

[a] Data taken from Ref. 148.

- The wrapping of Teflon tape on the outside of the joint between the glass stoppers and the neck of the volumetric flask did not reduce the evaporation rate.
- The average evaporation loss for 10-mL glass-stopper volumetric flasks filled with hexane and stored at ambient temperature, +3°C, and -15°C were 0.15, 0.06, and 0.02 mL/week, respectively.
- The evaporation rates of hexane and isooctane from prescription bottles were the lowest when compared to volumetric flasks, multivials, septum bottles, and small vials. Caps with a hard-liner backing underneath the Teflon liner gave the best seal. Figure A-16 gives the comparative evaporation rates of hexane and isooctane from different containers at ambient temperature.
- Solutions should be stored in a refrigerator or freezer to reduce solvent losses.
- Containers should be sealed tightly to reduce evaporation from around the cap seal.

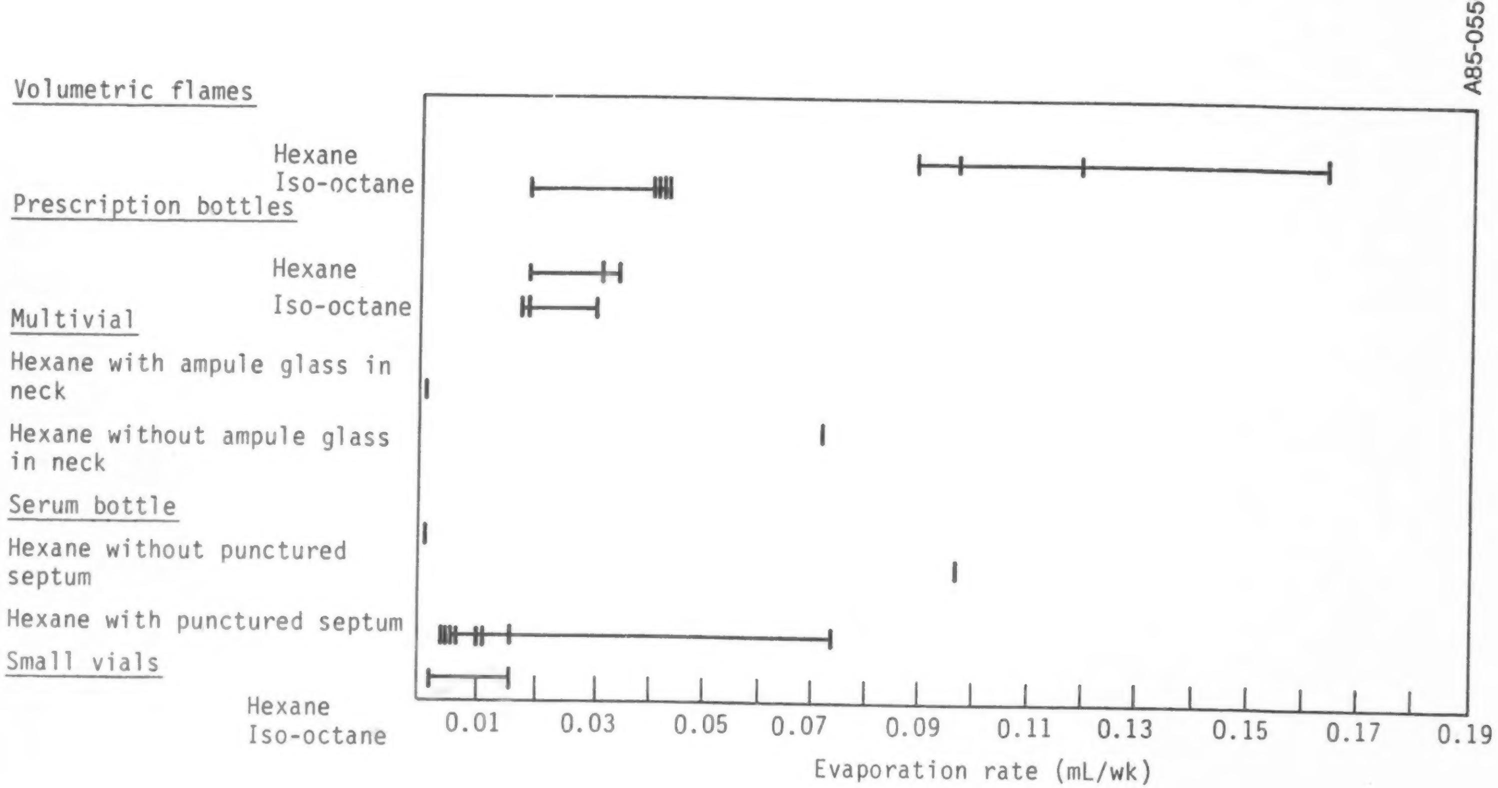

Figure A-16. Comparative evaporation rates (mL/wk) of hexane and isooctane from different containers at amibent temperature. The range of data for each container is plotted. Veritical lines represent different container volumes (i.e., volumetric flasks and prescription bottles) or different container/cap configurations (i.e., small vials). Data taken from Ref. 148.

- Decomposition of less than 10 percent was reported for 24 organochlorine pesticides and PCBs at four different storage conditions and for a 2-year period (Table A-41). Increase in compound concentration after a period of 10 months was attributed to solvent evaporation.

The authors also make the following recommendations for quality control practices:

- Primary reference standards should be stored in a desiccated container in the freezer.

- Concentrated stock solutions should be made fresh every year and should be stored in a freezer.

- The working solutions should be made fresh every 6 months (by dilution of stock solutions) and should be stored in a refrigerator when not in use. They should be replaced sooner if evaporation is evident or suspected.

TABLE A-41. ORGANOCHLORINE-IN-ISOOCTANE SOLUTIONS AND 2-YEAR STABILITY TEST RESULTS[a]

			Degradation at storage condition[c]			
Compound	Soln.[b]	Concentration (pg/μL)	Freezer -15°C	Refrigerator +3°C	Dark 24°C	Light 24°C
Aldrin	B	9.9	+	+	+	+
PCB-1016	E	100	+	+	+	+
PCB-1254	F	200	+	+	+	+
beta-BHC	C	16.2	+	+	+	+
gamma-BHC (lindane)	C	6.0	+	+	+	+
Chlordane (tech)	G	80.4	+	+	+	+
Chlordecone[d]	K	19.5	+	+	+	+
Chlordene	D	5.3	+	+	+	+
DCPA	B	15.1	+	+	+	+
4,4'-DDD	B	30.2	+	+	+	+
4,4'-DDE	A	22.0	+	+	+	+
2,4'-DDT	A	36.5	+	+	+	+
4,4'-DDT	A	47.8	+	+	+	+
Dieldrin	B	20.6	+	+	+	+
Endosulfan I	C	26.0	+	+	+	+
Endosulfan II	C	30.0	+	+	+	+
Endrin	C	50.0	+	+	+	+
HCB	C	5.0	+	+	+	+
Heptachlor	A	8.9	+	+	+	+
Heptachlor epoxide	A	15.0	+	+	+	+
1-Hydroxychlordene	D	15.8	+	+	+	+
Methoxychlor	F	71.4	+	+	+	+
Mirex	A	48.3	+	+	+	+
trans-Nonachlor	D	14.0	+	+	+	+
Oxychlordane	D	13.7	+	+	+	+
PCNB	A	7.8	+	+	+	+
Toxaphene	H	452	+	+	+	+

[a] Data taken from Ref. 149.

[b] Components of pesticide mixtures.

[c] Stable (+) or unstable (-).

[d] Solvent 1 percent methanol - benzene.

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APPENDIX B

METHOD 8080*
ORGANOCHLORINE PESTICIDES AND PCBs

1. Scope and Application

1.1 This method provides procedures for the determination of certain organochlorine pesticides and polychlorinated biphenyls (PCBs) in liquid and solid sample matrices. The following substances can be determined by this method:

Parameter	Storet No.	CAS No.
alpha-BHC	39337	319-84-6
beta-BHC	39338	319-85-7
gamma-BHC (Lindane)	39340	58-89-9
delta-BHC	34259	319-86-8
Heptachlor	39410	76-44-8
Aldrin	39330	309-00-2
Heptachlor epoxide	39420	1024-57-3
gamma-Chlordane	39350	57-74-9
Endosulfan I	34361	959-98-8
4,4'-DDE	39320	72-55-9
Dieldrin	39380	60-57-1
Endrin	39390	72-20-8
Endosulfan II	34356	33212-65-9
4,4'-DDD	39310	72-54-8
Endrin aldehyde	34366	7421-93-4
Kepone	39018	143-50-0
Endosulfan sulfate	34351	1031-07-8
4,4'-DDT	39300	50-29-3
4,4'-Methoxychlor	NA	72-43-5
Toxaphene	39400	8001-35-2
PCB-1016	34671	12674-11-2
PCB-1221	39488	1104-28-2
PCB-1232	39492	11141-16-5
PCB-1242	39496	53469-21-9
PCB-1248	39500	12672-29-6
PCB-1254	39504	11097-69-1
PCB-1260	39508	11096-82-5

NA -- Storet number not available at the time of protocol preparation.

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*Revised May 1986

1.2 This is a gas chromatographic (GC) method applicable to the determination of the substances listed above. When this method is used to analyze samples for any or all of these substances, compound identification should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm the measurements made with the primary column. Retention time information obtained on the two gas chromatographic columns is given in Table B-1.

1.3 The sample extraction and concentration steps in this method are those specified in Methods 3510 and 3520 for liquid matrices, and 3540 and 3550 for solid matrices.

1.4 Limitations: When both organochlorine pesticides and PCBs are present, fractionation of the extract by using silica gel chromatography is recommended. Toxaphene and chlordane, which are both multicomponent mixtures, cannot be satisfactorily separated by using silica gel chromatography and, therefore, may interfere with the quantification of certain organochlorine pesticides.

1.5 The method detection limit (MDL) for each parameter is listed in Table B-1. The MDLs for the components of a specific sample may differ from those listed in Table B-1 because the MDLs are dependent upon the nature of interferences in the sample matrix.

1.6 This method is restricted to use by or under the supervision of analysts experienced in the use of a gas chromatograph and in the interpretation of gas chromatograms.

2. Summary of Method

2.1 A measured volume or weight of sample (100 mL to 1 L for liquids, 10 g for solids) is extracted by using the appropriate sample extraction technique specified in Methods 3510, 3520, 3540, and 3550. Liquid samples are extracted at neutral pH with methylene chloride by using either a separatory funnel (Method 3510) or a continuous liquid-liquid extractor (Method 3520). Solid samples are extracted with hexane-acetone (1:1) by using either Soxhlet extraction (Method 3540) or sonication (Method 3550). After cleanup, the extract is analyzed by gas chromatography with electron capture detection.

2.2 The method provides a silica gel column fractionation procedure and an elemental sulfur removal procedure to aid in the elimination of interferences that may be encountered. A procedure for gel permeation chromatography cleanup is also provided for samples that contain high amounts of liquids and waxes.

3. Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may introduce discrete artifacts which result in elevated baselines causing

TABLE B-1. GAS CHROMATOGRAPHIC RETENTION TIMES AND METHOD DETECTION LIMITS FOR THE ORGANOCHLORINE PESTICIDES AND PCBs

	Retention Time (min)		MDL[c]	
Compound	DB-5[a]	SPB-608[b]	Liquid (µg/L)	Solid (µg/Kg)
alpha-BHC	12.29	9.46	0.035	1.9
beta-BHC	13.13	11.33	0.023	3.3
gamma-BHC (Lindane)	13.37	10.97	0.025	2.0
delta-BHC	14.14	12.73	0.024	1.1
Heptachlor	15.91	12.46	0.040	2.0
Aldrin	17.16	13.76	0.034	2.2
Heptachlor epoxide	18.60	15.98	0.032	2.1
gamma-Chlordane	19.48	16.70	0.037	1.5
Endosulfan I	19.94	17.40	0.030	2.1
4,4'-DDE	20.83	18.36	0.058	2.5
Dieldrin	20.91	18.60	0.044	I
Endrin	21.71	19.96	0.039	3.6
Endosulfan II	22.05	20.69	0.040	2.4
4,4'-DDD	22.38	20.53	0.050	4.2
Endrin aldehyde	22.75	21.90	0.050	1.6
Kepone	23.01	D	ND	ND
Endosulfan sulfate	23.64	22.54	0.035	3.6
4,4'-DDT	23.79	21.72	0.081	3.6
4,4'-Methoxychlor	25.94	24.90	0.086	5.7
Toxaphene	mr	mr	NA	NA
PCB-1016	mr	mr	0.54	57
PCB-1221	mr	mr	NA	NA
PCB-1232	mr	mr	NA	NA
PCB-1242	mr	mr	NA	NA
PCB-1248	mr	mr	NA	NA
PCB-1254	mr	mr	NA	NA
PCB-1260	mr	mr	0.90	70

Liquid: reagent water; Solid: sandy loam soil.
mr -- multiple peak response.
D -- degrades on column.
NA -- data not available.
ND -- not detected at 0.1 µg/L in liquid matrix or 10 µg/kg in solid matrix.
I -- unable to determine due to interference.

[a] Temperature program: 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; helium at 16 psi.

[b] Temperature program: 160°C (hold 2 min) to 290°C (hold 1 min) at 5°C/min; nitrogen at 20 psi.

[c] MDL is the method detection limit. MDL was determined from the analysis of seven replicate aliquots of each matrix processed through the entire analytical method (extraction, silica gel cleanup, and GC/EC analysis). MDL = $t_{(n-1,\ 0.99)} \times SD$ where $t_{(n-1,\ 0.99)}$ is the student's t value appropriate for a 99% confidence interval and a standard deviation with n-1 degrees of freedom, and SD is the standard deviation of the seven replicate measurements.

misinterpretation of gas chromatograms. These materials must therefore be demonstrated to be free from interferences under the conditions of the analysis by running method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Pesticide-grade or distilled-in-glass solvents are suitable for pesticide residue analysis without further purification. Each new batch of solvents should be checked for possible interferences as follows: concentrate to 1 mL the amount of solvent equivalent to the total volume to be used in the analysis. Inject 1 to 2 µL of the concentrate into a gas chromatograph equipped with an electron capture detector set at the lowest attenuation. If extraneous peaks are detected that are greater than 10 pg on-column, the solvent must be purified either by redistilling or by passing it through a column of highly activated alumina (acidic or basic alumina, activated at 300° to 400°C) or Florisil.

3.2 Interferences coextracted from the samples will vary considerably from waste to waste. While general cleanup techniques are provided as part of this method, unique samples may require additional cleanup approaches to achieve desired sensitivities.

3.3 Glassware must be scrupulously clean. Clean all glassware as soon as possible after use by rinsing with the last solvent used, followed by thorough washing of the glassware in hot, detergent-containing water. Rinse with tap water, distilled water, acetone, and finally pesticide-quality hexane. Heavily contaminated glassware may require treatment in a muffle furnace at 400°C for 15 to 30 min. Some high-boiling materials, such as PCBs, may not be eliminated by this treatment. Volumetric glassware should not be heated in a muffle furnace. Glassware should be sealed and stored in a clean environment immediately after drying or cooling to prevent any accumulation of dust or other contaminants. Store the glassware by inverting or by capping with aluminum foil.

A chromic acid wash is not satisfactory for the cleaning of glassware contaminated with organochlorine pesticides, most of the organochlorine pesticides are stable when exposed to chromic acid at room temperature for many days. Some pesticides such as heptachlor and aldrin react with chromic acid to form heptachlor epoxide and dieldrin.

3.4 Interferences by phthalate esters can pose a major problem in pesticide analysis. These materials elute in Fractions III and IV of the silica gel cleanup procedure. Their presence can usually be minimized by avoiding contact with any plastic materials.

3.5 The presence of elemental sulfur will result in large peaks and often mask the region from the solvent peak to the aldrin peak in the gas chromatogram. Several cleanup techniques for removal of sulfur have been reported (i.e., metallic mercury (2), activated copper (3), activated Raney Nickel (4), and tetrabutyl-ammonium sulfite (TBA) (5). The TBA procedure works well for the removal of elemental sulfur; for most organochlorine pesticides and PCBs, almost quantitative recoveries were obtained (Table B-2). Recoveries from actual samples may be lower. Since the recovery of endrin

TABLE B-2. RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs USING THE TBA PROCEDURE (STANDARDS ONLY, IN HEXANE)

Compound	Percent Recovery: Without sulfur added[a]	Percent Recovery: With sulfur added[b]
alpha-BHC	110	103
beta-BHC	106	99
gamma-BHC (Lindane)	112	108
delta-BHC	100	86
Heptachlor	103	97
Aldrin	104	99
Heptachlor epoxide	107	101
gamma-Chlordane	98	100
Endosulfan I	108	106
4,4'-DDE and Dieldrin	91	103
Endrin	87	89
Endosulfan II	99	99
4,4'-DDD	103	106
Endrin aldehyde	10	8
Kepone	107	63
Endosulfan sulfate	94	93
4,4'-DDT	86	90
4,4'-Methoxychlor	85	68
Toxaphene	93	83
PCB-1016	108	88
PCB-1260	87	68

[a] The values given are the averages of two determinations.
[b] Single determination.

aldehyde is drastically reduced, this compound must be determined prior to sulfur cleanup.

3.6 Waxes and lipids can be removed by gel permeation chromatography (6). Extracts containing high amounts of lipids are viscous and may even solidify at room temperature.

4. Safety

4.1 The toxicity and carcinogenicity of most of the compounds to be determined by this method have not been precisely defined; therefore, each compound should be treated as a potential health hazard, and exposure of personnel to these compounds must be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in chemical analysis.

4.2 Primary standards of pesticides and PCBs should be prepared in a hood. A NIOSH/MESA-approved toxic gas respirator should be worn when the analyst handles high concentrations of these toxic compounds.

5. Apparatus and Materials

5.1 Glassware (see also Methods 3510, 3520, 3540 and 3550 specifications)

5.1.1 Drying column: chromatographic column, approximately 150 mm long x 25 mm ID, with coarse frit filter disc.

5.1.2 Kuderna-Danish (K-D) apparatus

5.1.2.1 Concentrator tube: 10 mL, graduated. Calibration must be checked at the 1.0- and 10.0-mL level. Ground glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

5.1.2.2 Evaporative flask: 50 mL. Attach to concentrator tube with springs.

5.1.2.3 Snyder column: Three-ball macro (Kontes K503000-0121 or equivalent).

5.1.2.4 Boiling chips: Approximately 10/40 mesh. Heat to 400°C for 30 min or Soxhlet-extract with methylene chloride.

5.2 Water bath: heated, with concentric ring cover, capable of temperature control (±2°C). The bath should be used in a hood.

5.3 Gel permeation chromatography (GPC) cleanup device

5.3.1 Automated system

5.3.1.1 Gel permeation chromatograph, Analytical Biochemical Labs, Inc., GPC Autoprep 1002, or equivalent, including:

5.3.1.2 25 mm ID by 600-700 mm glass column packed with 70 g Bio-Beads SX-3.

5.3.1.3 Syringe, 10 mL with luer lok fitting.

5.3.1.4 Syringe filter holder and filters -- stainless steel and TFE, Gelman 4310, or equivalent.

5.3.2 Manual system assembled from parts (6)

5.3.2.1 24 mm ID by 600-700 mm heavy-wall glass column packed with 70g Bio-Beads SX-3.

5.3.2.2 Pump: Altex Scientific, Model No. 1001A, semipreparative, solvent metering system. Pump capacity: 28 mL/min.

5.3.2.3 Detector: Altex Scientific, Model No. 153, with 254 nm UV source and 8-uL semipreparative flowcell (2-mm pathlengths).

5.3.2.4 Microprocessor/controller: Altex Scientific, Model No. 420, Microprocessor System Controller, with extended memory.

5.3.2.5 Injector: Altex Scientific, Catalog No. 201-56, sample injection valve, Tefzel, with 10-mL sample loop.

5.3.2.6 Recorder: Linear Instruments, Model No. 385, 10-in. recorder.

5.3.2.7 Effluent Switching Valve: Teflon slider valve, 3-way with 0.060 in. ports.

5.3.2.8 Supplemental Pressure Gauge with Connecting Tee: U.S. Gauge, 0 to 200 psi, stainless steel. Installed as a "downstream" monitoring device between column and detector. Flowrate was typically 5 mL/min of methylene chloride. Recorder chart speed was 0.50 cm/min.

5.4 Gas chromatograph: An analytical system complete with gas chromatograph suitable for on-column and split/splitless injection, and all required accessories including syringes, analytical columns, gases, electron capture detector, and recorder/integrator or data system.

5.4.1 Column 1: 30 m x 0.25 or 0.32 mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5 or equivalent).

5.4.2 Column 2: 30 m x 0.25 mm ID fused-silica capillary column SPB-608 (Supelco, Inc.), 0.25 pm coating thickness.

5.5 Chromatographic column for silic gel: 200 mm x 11 mm ID, glass column.

6. Reagents

6.1 Reagent water: Reagent water is defined as water in which an interferent is not observed at the MDL of the parameters of interest.

6.2 Preservatives

6.2.1 Sodium hydroxide (ACS): 10 N in distilled water.

6.2.2 Sulfuric acid (ACS) (1 + 1): mix equal volumes of concentrated sulfuric acid with distilled water.

6.3 Acetone, hexane, isooctane, methylene chloride: pesticide quality or equivalent.

6.4 Ethyl acetate, methanol, 2-propanol: nanograde.

6.5 Sodium sulfate (ACS): granular, anhydrous. Purify by heating at 400°C for 4 hours in a shallow tray.

6.6 Silica gel PR grade (100/200 mesh): before use, activate at least 16 hours at 130° to 140°C. Deactivate with water (3.3 percent by weight). Equilibrate for 1 hour after addition of the water.

6.7 Tetrabutyl ammonium sulfite reagent: a solution of 3.39 g (0.01 mol) tetrabutyl ammonium hydrogen sulfate in 100 mL of water is extracted with three 20-mL portions of hexane (to remove impurities), and then 25 g anhydrous sodium sulfite (analytical grade) are added.

6.8 Stock standard solutions (1.0 µg/µL): stock standard solutions can be prepared from pure standard materials or can be purchased as certified solutions. The NBS Office of Standard Reference Materials developed the Standard Reference Material 1583, chlorinated pesticides in 2,2,4-trimethylpentane certified for five chlorinated pesticides: gamma-BHC, delta-BHC, aldrin, 4,4'-DDE, and 4,4'-DDT. An uncertified concentration is provided for heptachlor epoxide. The concentrations range from approximately 0.8 to 1.9 µg/g; the corresponding concentrations in µg/mL at 23°C are also provided.

6.8.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure compound. Dissolve the compound in isooctane and dilute to volume in a 10-mL volumetric flask. If compound purity is 96 percent or greater, the weight can be used without correction to calculate the concentration of the stock standard.

6.8.2 Beta-BHC, dieldrin, and Kepone are not adequately soluble in isooctane; methanol, acetone, or benzene should therefore be used for the preparation of the stock standard solutions of these compounds.

6.8.3 Transfer the stock standard solutions into sealed screw-cap bottles or ground-glass-stoppered reagent bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation especially just prior to preparing calibration standards.

6.9 Corn Oil Solution: 200 mg/mL in methylene chloride.

7. Sample Collection, Preservation, and Handling

7.1 Grab samples must be collected in appropriately cleaned glass containers, and the sampling bottles must not be prewashed with the sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be free of tygon and other potential sources of contamination.

7.2 Liquid samples must be iced or refrigerated from the time of collection until extraction. Chemical preservatives should not be used in the field unless more than 24 hours will elapse before delivery to the laboratory. If the samples will not be extracted within 48 hours of collection, the sample should be adjusted to a pH range of 6.0 to 8.0 with sodium hydroxide or sulfuric acid.

7.3 Stability of the organochlorine pesticides and PCBs in soil has not been systematically investigated. Storage of soil samples at room temperature should be avoided since degradation of some organochlorine pesticides has been reported to occur (7). Losses of lindane and aldrin, after incubation of spiked soil samples at 25°C under aerobic conditions, were noticeable 7 days after the initiation of the experiment, and only 35 to 40 percent of the two pesticides were found at day 30 of the experiment. Deep-freezing at -10°C or -20°C appears to be the most suitable method for storage of solid matrices since it has the widest range of application, causes the least changes in the samples, and makes the addition of preservatives unnecessary.

7.4 All samples must be extracted within 7 days of collection and must be completely analyzed within 30 days of extraction.

8. Procedures

8.1 Sample Preparation

8.1.1 Extraction. Extract water samples at a neutral pH with methylene chloride as a solvent by using a separatory funnel (Method 3510) or a continuous liquid-liquid extractor (Method 3520). Extract solid samples with hexane:acetone (1:1) by using either the Soxhlet extraction (Method 3540) or sonication procedures (Method 3550).

8.1.2 Spiked samples are used to verify the applicability of the chosen extraction technique to each new sample type. Each sample must be spiked with the compounds of interest to determine the percent recovery and the limit of detection for that sample. Spiking of water samples should be performed by adding appropriate amounts of pesticide and PCB compounds, dissolved in methanol, to the water sample immediately prior to extraction. After addition of the spike, mix the samples manually for 1 to 2 min. Typical spiking levels for water samples are 1 to 10 µg/L for samples in which pesticides and PCBs were not detected, and 2 to 5 times the background level in those cases where pesticides and PCBs are present. Spiking of solid samples should be performed by adding appropriate amounts of pesticides and PCB compounds, which are dissolved in methanol, to the solid samples. The solid sample should be wet prior to the addition of the spike (at least 20 percent moisture) and should be mixed thoroughly with a glass rod to homogenize the material. Allow the spike to equilibriate with the solid for 1 hour at room temperature prior to extraction. Transfer the whole portion that was spiked with the test compounds to the extraction thimble for Soxhlet extraction (Method 3540) or proceed with the sonication extraction in the case of Method 3550.

8.2 Cleanup/Fractionation

8.2.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. If PCBs are known to be present in the sample, then the silica gel fractionation is recommended. Elemental sulfur, which may appear in certain sediments and industrial wastes and which interferes with the electron capture gas chromatography of certain pesticides, can be removed by the technique described in Section 8.3.

8.2.2 Silica gel column cleanup/fractionation

8.2.2.1 Place a weight of silica gel (normally 20 g) activated for at least 16 hours at 130°C into a glass jar and deactivate it with reagent water to bring the moisture content to 3.3 percent. Mix the contents of the glass jar thoroughly and equilibrate for 6 hours. Store the deactivated silica gel in a sealed glass jar inside a desiccator. Transfer a 3-g portion into an 11-mm ID glass column and top it with 2 to 3 cm of anhydrous sodium sulfate.

8.2.2.2 Add 10 mL hexane to the top of the column to wet and rinse the sodium sulfate and silica gel. Just prior to exposure of the sodium sulfate layer to air, stop the hexane eluate flow by closing the stopcock on the chromatographic column. Discard the eluate.

8.2.2.3 Transfer the sample extract (2 mL) onto the column. Rinse the extract vial twice with 1 to 2 mL hexane and add each rinse to the column. Elute the column with 80 mL hexane (Fraction I) at a rate of about 5 mL/min. Remove the collection flask and set it aside for later concentration. Elute the column with 50 mL hexane (Fraction II) and collect the eluate. Perform a third elution with 15 mL methylene chloride (Fraction III) and a fourth one with 50 mL ethyl acetate (Fraction IV). The elution patterns for the organochlorine pesticides and PCBs are shown in Table B-3. If it is known that Kepone is not present, then Fractions III and IV may be combined. This should be done only in those cases where matrix coextractants do not interfere with the analysis of pesticides present in Fraction III.

8.2.2.4 Concentrate the fractions as described in Methods 3510, 3520, 3540, and 3550. Methylene chloride is exchanged for hexane (Fraction III), and the ethyl acetate in Fraction IV is exchanged for benzene-methanol (98:2). The exchange step is performed by adding 50 mL of the solvent/solvent mixture specified above and by concentrating the extract using a Kuderna-Danish apparatus. When the apparent volume of the liquid reaches 1 mL, remove the Kuderna-Danish apparatus and allow it to drain and cool for at least 10 min. Proceed with the nitrogen blowdown technique. Losses of the more volatile organochlorine pesticides (lindane, heptachlor,

TABLE B-3. DISTRIBUTION AND PERCENT RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs IN THE SILICA GEL COLUMN FRACTIONS [a,b,c,d,e]

Compound	Fraction I		Fraction II		Fraction III		Fraction IV		Total Recovery	
	Conc. 1	Conc. 2	Conc. 1	Conc. 2	Conc. 1	Conc. 2	Conc. 1	Conc. 2	Conc. 1	Conc. 2
alpha-BHC[f]					82 (1.7)	74 (8.0)			82 (1.7)	74 (8.0)
beta-BHC					107 (2.1)	98 (12.5)			107 (2.1)	98 (12.5)
gamma-BHC					91 (3.6)	85 (10.7)			91 (3.6)	85 (10.7)
delta-BHC					92 (3.5)	83 (10.6)			92 (3.5)	83 (10.6)
Heptachlor	109 (4.1)	118 (8.7)							109 (4.1)	118 (8.7)
Aldrin	97 (5.6)	104 (1.6)							97 (5.6)	104 (1.6)
Heptachlor epoxide					95 (4.7)	88 (10.2)			95 (4.7)	88 (10.2)
Endosulfan I					95 (5.1)	87 (10.2)			95 (5.1)	87 (10.2)
4,4'-DDE	86 (5.4)	94 (2.8)							86 (5.4)	94 (2.8)
Dieldrin					96 (6.0)	87 (10.6)			96 (6.0)	87 (10.6)
Endrin					85 (10.5)	71 (12.3)			85 (10.5)	71 (12.3)
Endosulfan II					97 (4.4)	86 (10.4)			97 (4.4)	86 (10.4)
4,4'-DDD[f]					102 (4.6)	92 (10.2)			102 (4.6)	92 (10.2)
Endrin aldehyde					81 (1.9)	76 (9.5)	29 (4.2)	33 (4.7)	110 (3.0)	109 (5.8)
Kepone							41 (7.9)	73 (12.0)	41 (7.9)	73 (12.0)
Endosulfan sulfate					93 (4.9)	82 (9.2)			93 (4.9)	82 (9.2)
4,4'-DDT[f]			86 (13.4)	73 (9.1)	15 (18.7)	8.7 (15.0)			101 (5.3)	82 (23.7)
4,4'-Methoxychlor					99 (9.9)	82 (10.7)	9.0 (4.8)	5.7 (3.0)	108 (10.0)	88 (5.8)
PCB-1016	86 (4.0)	87 (6.1)							86 (4.0)	87 (6.1)
PCB-1260	91 (4.1)	95 (5.0)							91 (4.1)	95 (5.0)
Technical chlordane	14 (5.5)	22 (5.3)	19 (6.8)	39 (3.6)	29 (5.0)	37 (5.1)			62 (3.3)	98 (1.9)
Toxaphene			15 (2.4)	17 (1.4)	73 (9.4)	84 (10.7)			88 (12.0)	101 (10.1)

[a] Eluant composition:
Fraction I - 80 mL hexane.
Fraction II - 50 mL hexane.
Fraction III - 15 mL methylene chloride.
Fraction IV - 50 mL ethyl acetate.

[b] Concentration 1 is 0.5 µg per column for BHCs, heptachlor, aldrin, heptachlor epoxide, endosulfan I; 1.0 µg per column for dieldrin, Kepone, endosulfan II, 4,4'-DDT, endrin aldehyde, 4,4'-DDD, 4,4'-DDE, endrin, and endosulfan sulfate; 5 µg per column for 4,4'-methoxychlor and technical chlordane; 10 µg per column for toxaphene, PCB-1016,and PCB-1260.

[c] For concentration 2 the amounts spiked are 10 times as high as those for concentration 1.

[d] The values given represent the average recoveries of three determinations; the numbers in parentheses are the standard deviations; recovery cutoff point is 5 percent.

[e] Data obtained with standards, as indicated in footnotes b and c, dissolved in 2 mL hexane.

[f] It has been found that because of batch to batch variation in the silica gel material these compounds cross over in two fractions, and the amounts recovered in each fraction are difficult to reproduce.

aldrin) have been reported (8) when sample extracts were concentrated to a volume of 0.5 mL or less by a stream of air or nitrogen; losses increased as the residual volume approached dryness and were relatively greater when smaller amounts of pesticides were present.

8.3 Elemental Sulfur Removal -- Shake the hexane extract (2 mL) with 2-propanol (1 mL) and the TBA-sulfite reagent (1 mL) for at least 1 min. Add approximately 100 mg crystalline sodium sulfite. If the sodium sulfite disappears, more sodium sulfite is added in 100-mg portions until a solid residue remains after repeated shaking. Water (5 mL) is added, and the test tube is shaken for another minute; this process is followed by centrifugation. Finally, the hexane layer is transferred to another vial for analysis.

8.4 Gel Permeation Chromatography Cleanup

8.4.1 Packing the column: Place 70 g of Bio-Beads SX-3 in a 400-mL beaker, cover the beads with methylene chloride, and allow them to swell overnight (before packing the columns). Transfer the swelled beads to the column and pump methylene chloride through the column from bottom to top at 5.0 mL/min. After approximately 1 hour, adjust the pressure on the column to 7 to 10 psi and pump an additional 4 hours to remove all air from the column. Adjust the column pressure periodically, as required, to maintain 7 to 10 psi.

8.4.2 Calibration of the column: Load 5 mL of the corn oil solution into sample loop No. 1, and 5 mL of the pesticide-PCB standard into loop No 2. Inject the corn oil and collect 10 mL fractions (i.e., change fraction at 2-min intervals) for 36 min. Inject the pesticide-PCB standard and collect 15-mL fractions for 60 min. Determine the corn oil elution pattern by evaporation of each fraction to dryness with a gravimetric determination of the residue. Analyze the pesticide-PCB fractions by gas chromatography and plot the concentration of each component in each fraction versus total eluant volume (or time) from the injection points. Choose a "dump time" which allows >85 percent removal of the corn oil and >85 percent recovery of the pesticide-PCB compounds. Choose the "collect time" that will extend to at least 10 min after the elution of the pesticide-PCB compounds. Wash the column for at least 15 min between samples. Typical parameters selected are the dump time, 30 min (150 mL), the collect time, 36 min (180 mL), and the wash time, 15 min (75 mL). The column can also be calibrated by the use of a 254 nm UV detector in place of gravimetric and GC analyses of fractions. Measure the peak areas at various elution times to determine appropriate fractions.

The SX-3 Bio-Beeds column may be reused for several months even if discoloration occurs. System calibration usually remains constant over this period of time if the column flowrate remains constant.

8.4.3 Prefilter or load all extracts via the filter holder to avoid particulates that might cause flow stoppage. Load one 5.0-mL aliquot of the extract onto the GPC column. Use sufficient clean solvent after the extract to transfer the entire aliquot into the loop. Between extracts, purge the sample loading tubing thoroughly with clean solvent. Process the extracts by using the dump, collect, and wash parameters determined from the calibration and collect the cleaned extracts in 250-mL amber bottles. Concentrate the extracts as described in Section 8.2.2.4.

9. Gas Chromatography

9.1 Gas chromatographic conditions. The recommended gas chromatographic columns and operating conditions for the GC/EC instrument are as follows:

- Column 1: temperature program from 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; carrier gas helium at 16 psi; injector temperature 225°C; detector temperature 300°C.
- Column 2: temperature program from 160°C (hold 2 min) to 290°C (hold 1 min) at 5°C/min; carrier gas nitrogen at 20 psi; injector temperature 225°C; detector temperature 300°C.

9.2 Table B-1 gives the retention times and the MDLs that can be achieved by this method for the organochlorine pesticides and PCBs. Examples of the separations achieved with the DB-5 fused-silica capillary column are shown in Figures B-1 to B-10.

9.3 Calibration

9.3.1 Establish the gas chromatographic operating parameters equivalent to those indicated in Section 9.1. The gas chromatographic system can be calibrated by using the external standard technique (Section 9.3.2) or the internal standard technique (Section 9.3.3).

9.3.2 External standard calibration procedure

9.3.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding volumes of one or more stock standards to a volumetric flask and by diluting to volume with isooctane. One of the external standards should be at a concentration near but above the MDL. The other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

9.3.2.2 By using injections of 1 to 2 µL of each calibration standard, tabulate area responses against the amounts injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the amount injected, defined as the calibration factor (CF), can be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 15 percent over the working range, linearity through the origin can be assumed, and the average calibration factor can be used in place of a calibration curve.

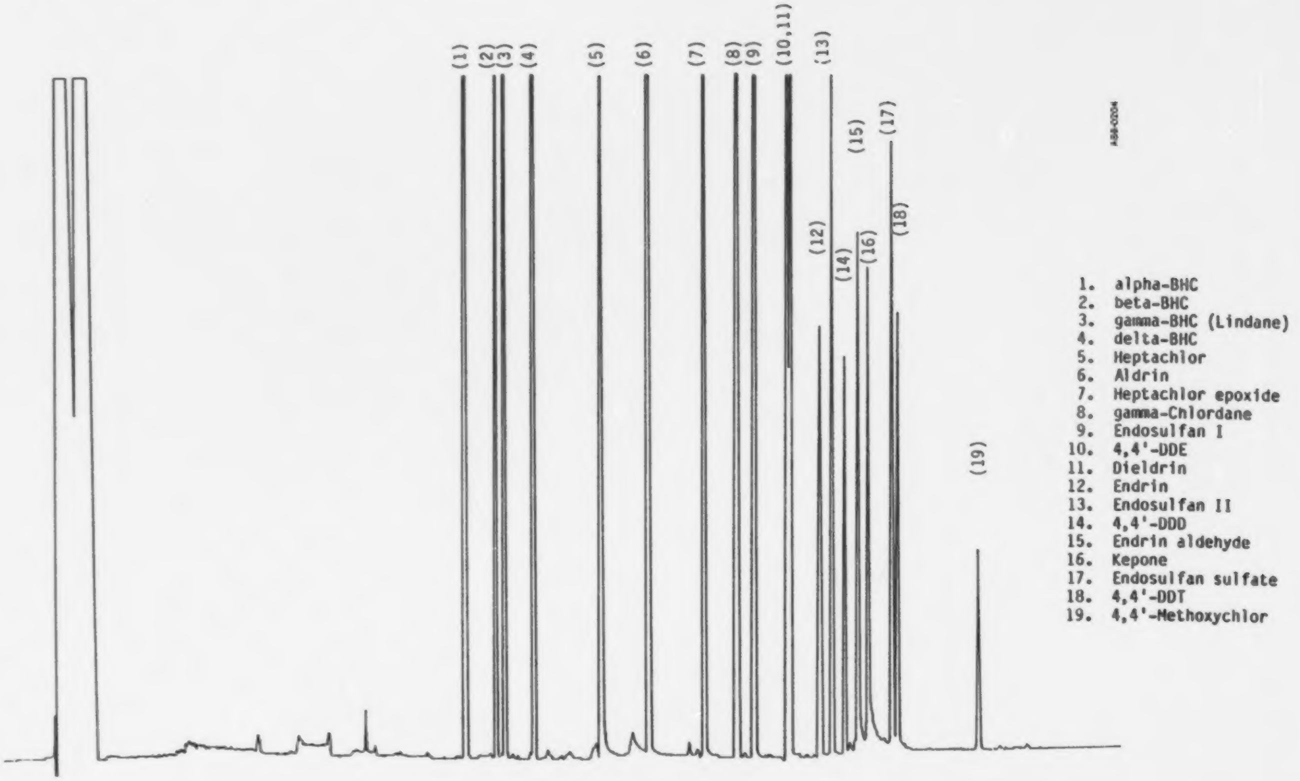

Figure B-1. Gas chromatogram of the organochlorine pesticide standard; column: 30 m x 0.25 mm ID DB-5 fused-silica capillary. Temperature program: 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; carrier gas He at 16 psi.

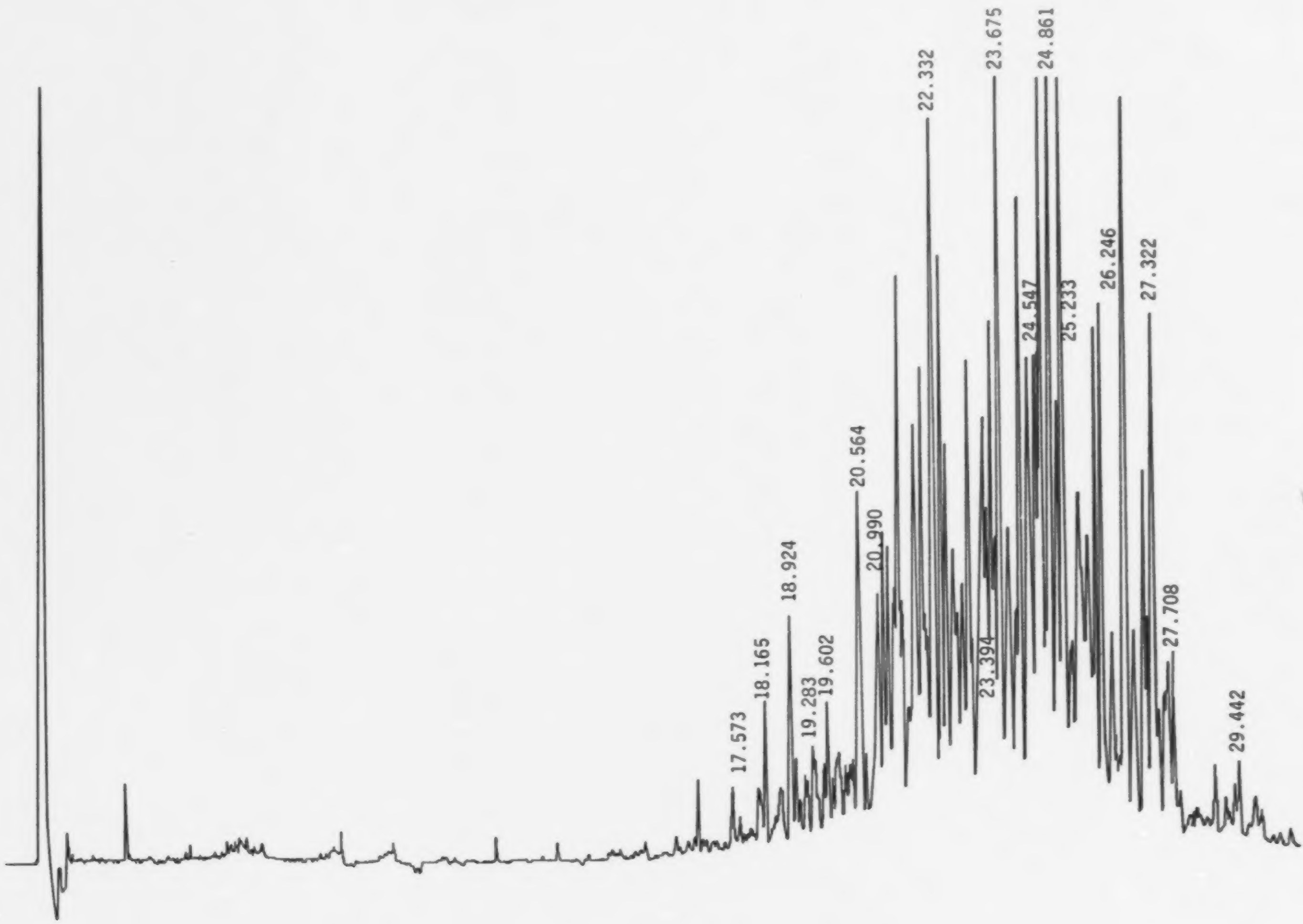

Figure B-2. Gas chromatogram of the toxaphene standard; column: 30 m x 0.25 mm ID DB-5 fused-silica capillary. Temperature program: 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; carrier gas He at 16 psi.

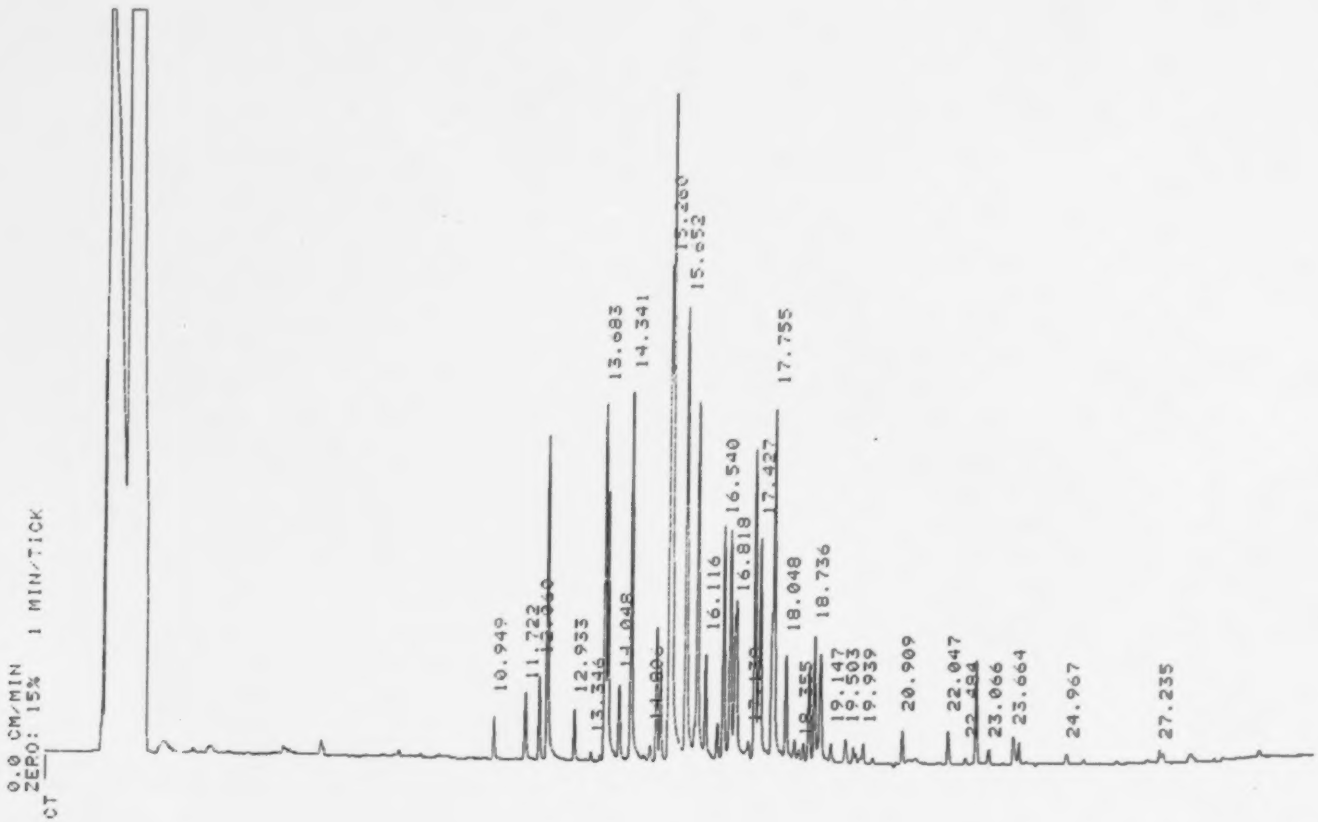

Figure B-3. Gas chromatogram of the PCB-1016 standard; column: 30 m x 0.25 mm ID DB-5 fused-silica capillary. Temperature program: 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; carrier gas He at 16 psi.

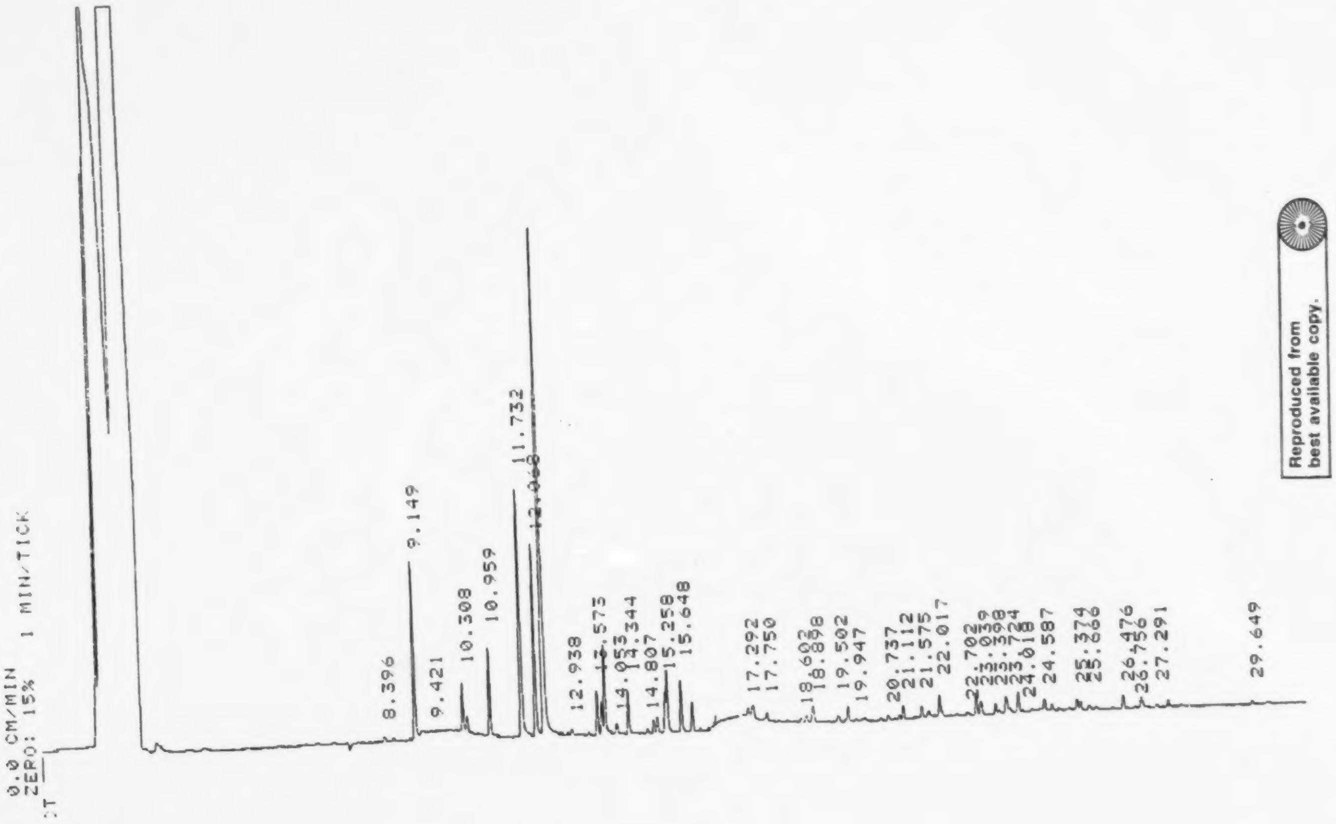

Figure B-4. Gas chromatogram of the PCB-1221 standard; column: 30 m x 0.25 mm ID DB-5 fused-silica capillary. Temperature program: 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; carrier gas He at 16 psi.

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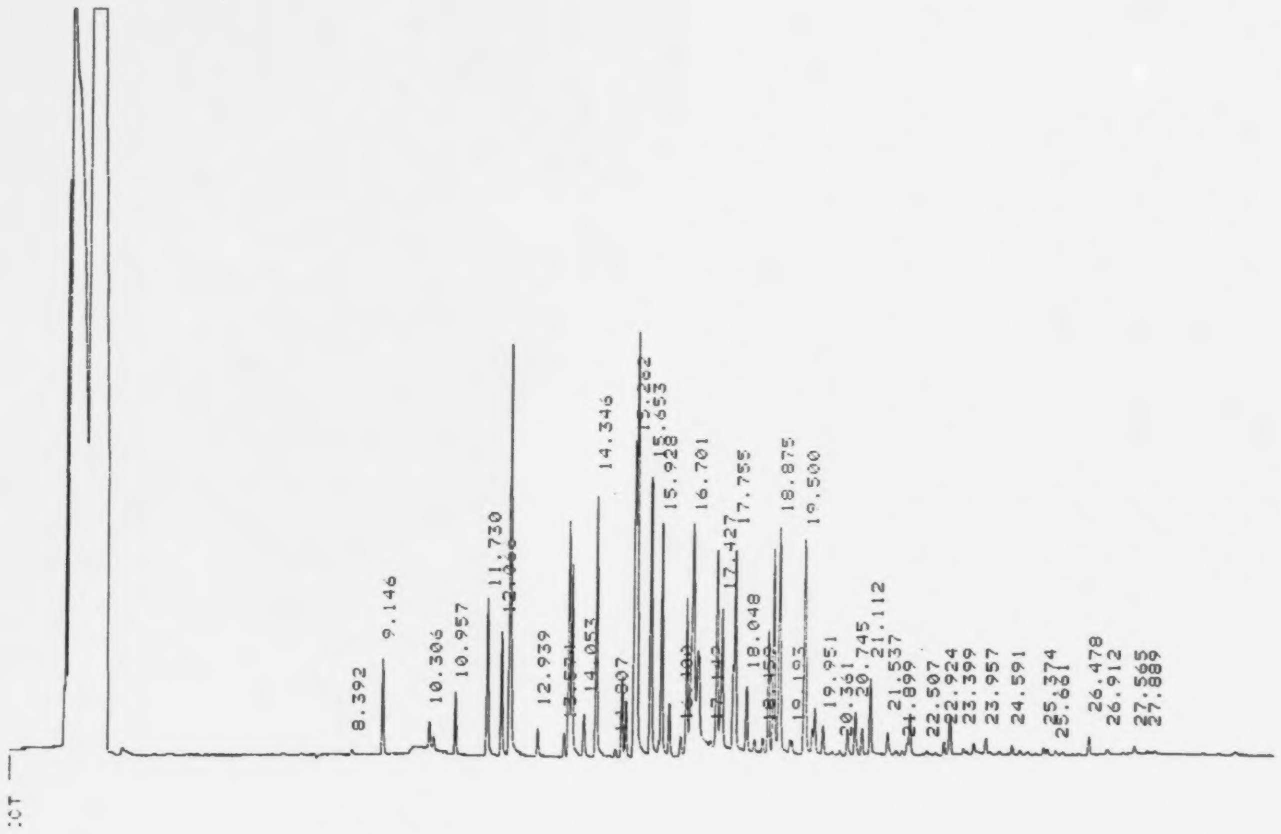

Figure B-5. Gas chromatogram of the PCB-1232 standard; column: 30 m x 0.25 mm ID DB-5 fused-silica capillary. Temperature program: 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; carrier gas He at 16 psi.

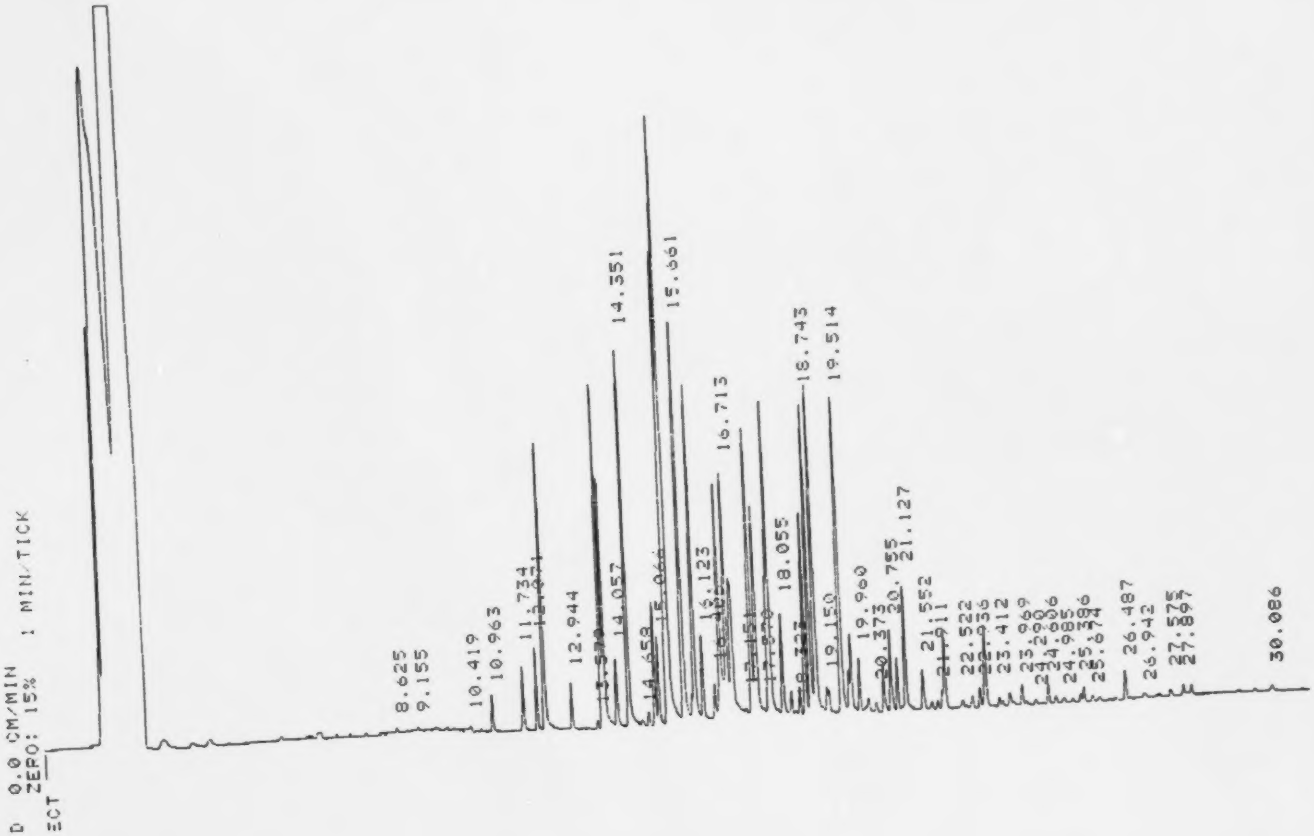

Figure B-6. Gas chromatogram of the PCB-1242 standard; column: 30 m x 0.25 mm ID DB-5 fused-silica capillary. Temperature program: 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; carrier gas He at 16 psi.

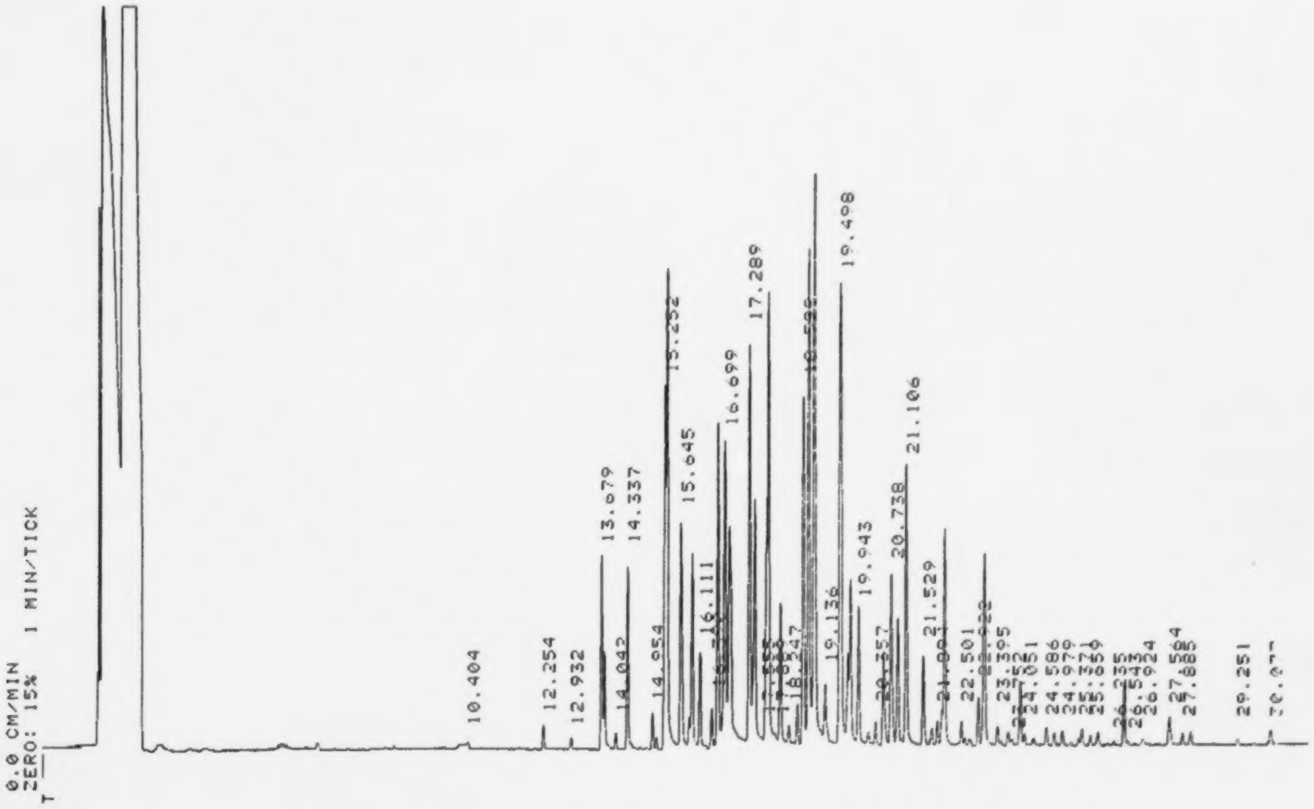

Figure B-7. Gas chromatogram of the PCB-1248 standard; column: 30 m x 0.25 mm ID DB-5 fused-silica capillary. Temperature program: 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; carrier gas He at 16 psi.

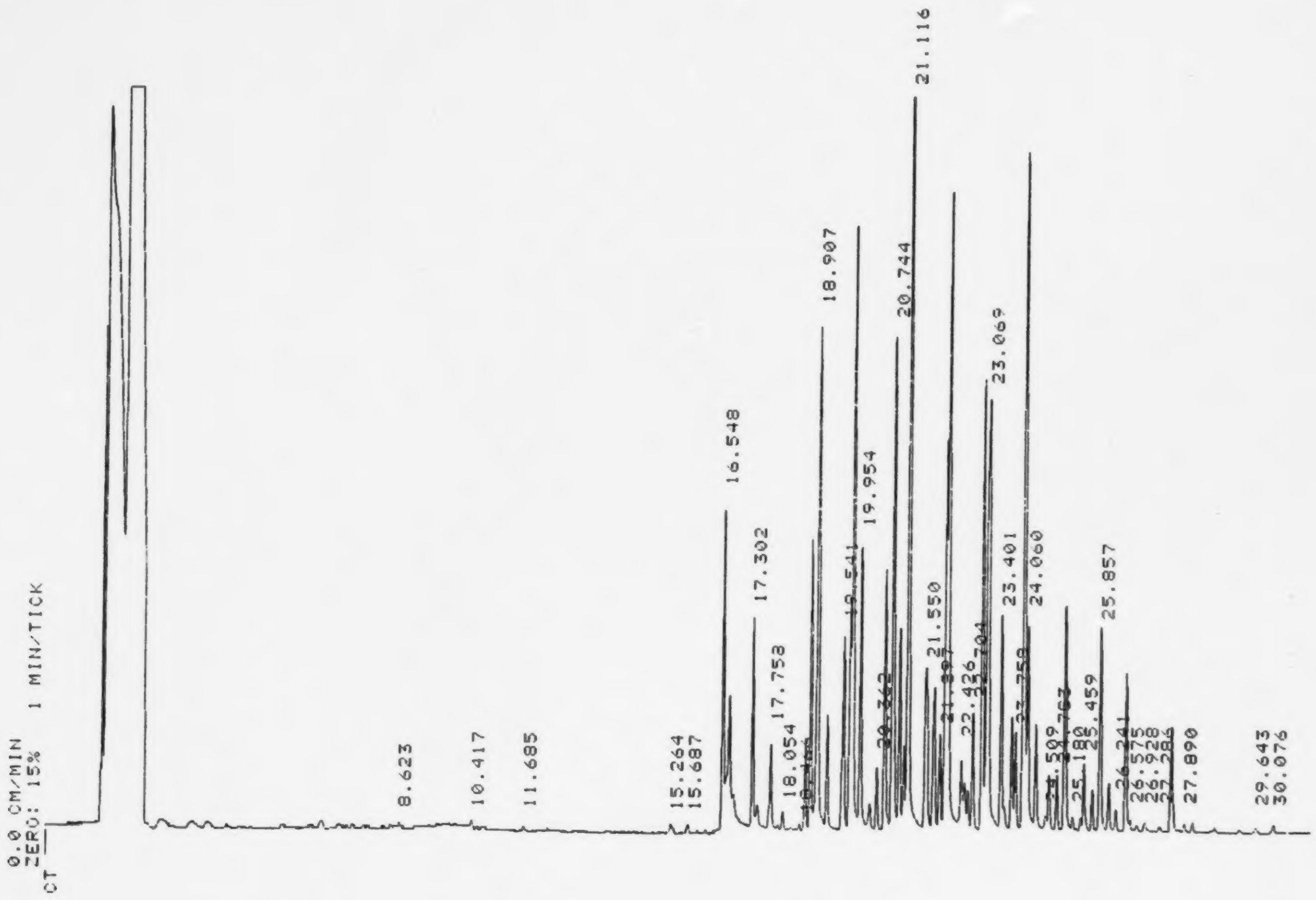

Figure B-8. Gas chromatogram of the PCB-1254 standard; column: 30 m x 0.25 mm ID DB-5 fused-silica capillary. Temperature program: 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; carrier gas He at 16 psi.

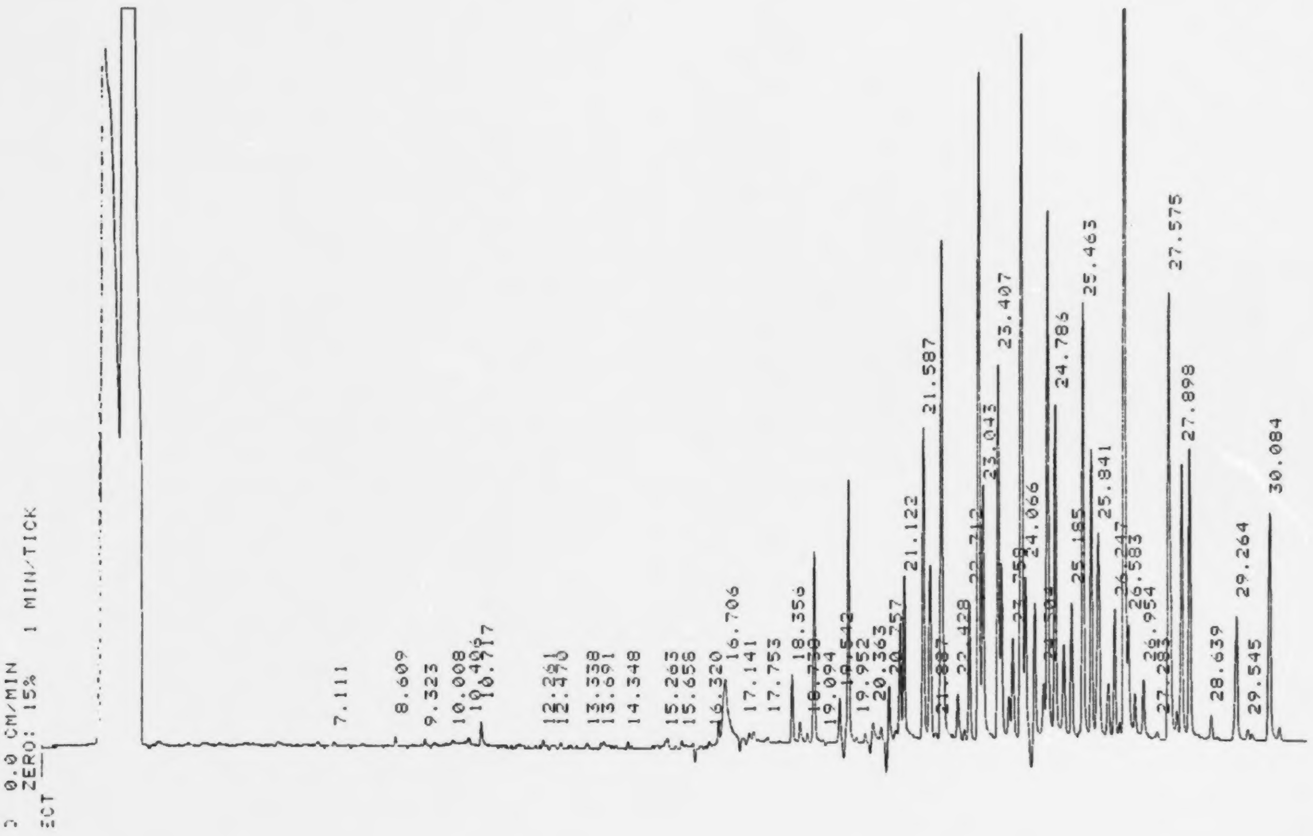

Figure B-9. Gas chromatogram of the PCB-1260 standard; column: 30 m x 0.25 mm ID DB-5 fused-silica capillary. Temperature program: 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; carrier gas He at 16 psi.

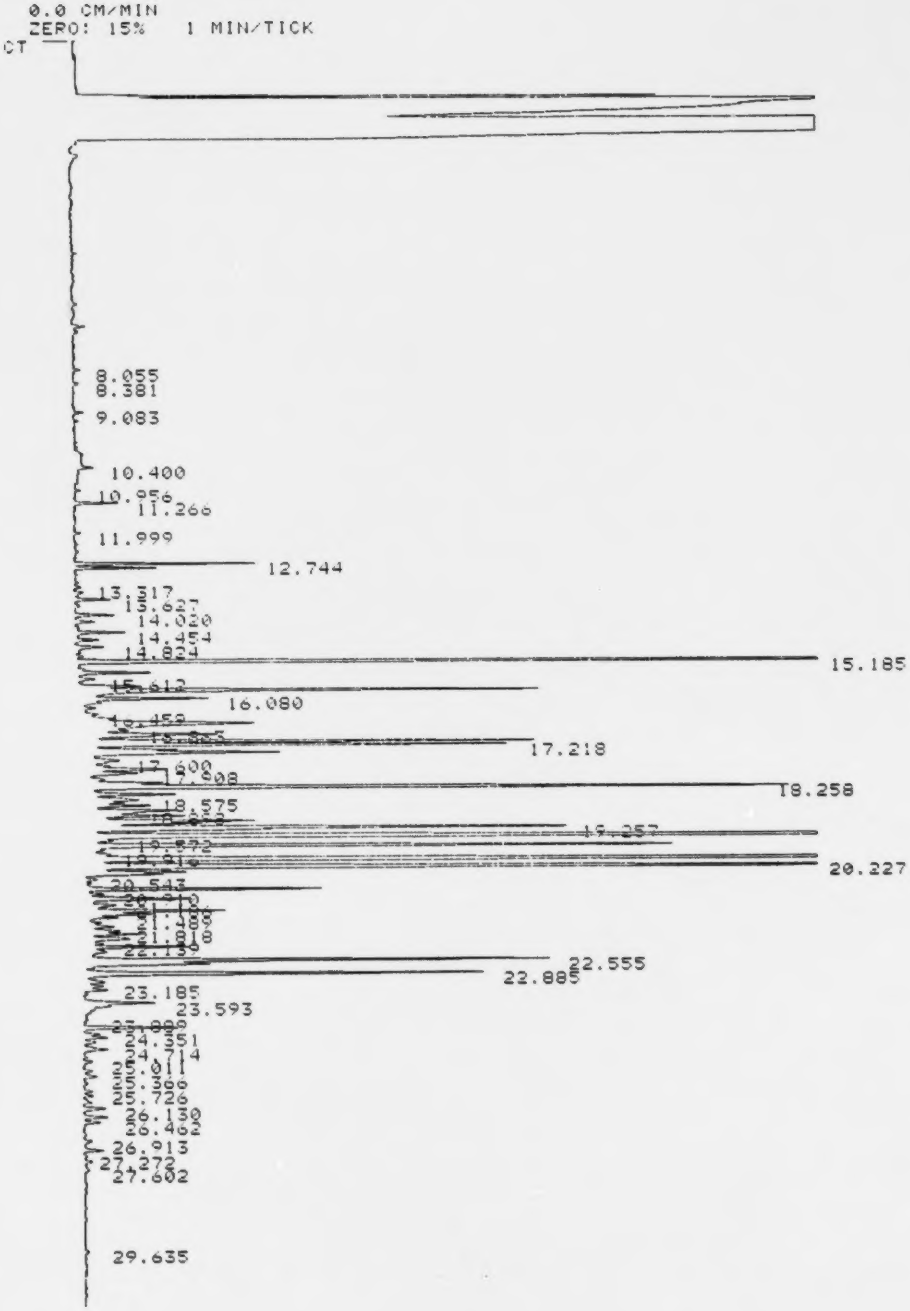

Figure B-10. Gas chromatogram of the technical chlordane standard; column: 30 m x 0.25 mm ID DB-5 fused-silica capillary. Temperature program: 100°C (hold 2 min) to 160°C at 15°C/min, then at 5°C/min to 270°C; carrier gas He at 16 psi.

9.3.2.3 The working calibration curve or the calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±20 percent, the test must be repeated with a fresh calibration standard. Alternatively, a new calibration curve or calibration factor may be prepared for that parameter.

9.3.2.4 For multicomponent mixtures (toxaphene and PCBs), match retention times of peaks in the standards with peaks in the sample. Quantify 8 to 10 major peaks, add peak areas, and calculate as total response in the sample versus total response in the standard.

9.3.2.5 Technical chlordane is a mixture of alpha-chlordane (13 percent), gamma-chlordane (18 percent), heptachlor (8 percent), chlordene (19 percent), and other compounds from the chlorination of chlordene. The sum of the concentrations of alpha-chlordane and gamma-chlordane can be used to estimate the concentration of chlordane.

$$C_{\text{technical}} = \frac{C_{\text{alpha}} + C_{\text{gamma}}}{0.31}$$

where $C_{\text{technical}}$ = estimated concentration of technical chlordane
C_{alpha} = measured concentration of alpha-chlordane
C_{gamma} = measured concentration of gamma-chlordane.

9.3.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard applicable to all samples can be suggested.

9.3.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with isooctane. One of the calibration standards should be at a concentration near but above the MDL. The other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

9.3.3.2 By using injections of 1 to 2 µL of each calibration standard, tabulate the area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

$$RF = (A_s C_{is})/(A_{is} C_s)$$

where:

A_s = Response for the parameter to be measured
A_{is} = Response for the internal standard
C_{is} = Concentration of the internal standard in ng/µL
C_s = Concentration of the parameter to be measured in ng/µL.

If the RF value over the working range is constant with less than ±15 percent relative standard deviation, the RF can be assumed to be invariant, and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is} against RF.

9.3.3.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standard. If the response for any parameter varies from the predicted response by more than 20 percent, the test must be repeated with a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

9.4 Identify compounds in the sample by comparing the retention times of the peaks in the sample chromatogram with those of the peaks in standard chromatograms. The retention time window used to make identifications is based upon measurements of actual retention time variations over the course of 10 consecutive injections (Table B-4). Three times the standard deviation of a retention time window can be used to calculate a suggested window size.

9.5 If the response of a peak exceeds the working range of the system, dilute the extract and reanalyze.

10. Quality Control

10.1 Before processing any samples, the analyst should demonstrate through the analysis of a distilled water method blank that all glassware and reagents are interference-free. Each time a set of samples is extracted or there is a change in reagents, a method blank should be processed as a safeguard against laboratory contamination. The blank samples should be carried through all stages of the sample preparation, cleanup, and analysis steps.

10.2 Standard quality assurance practices should be used with this method. Field replicates should be collected to validate the precision of the sampling technique. Laboratory replicates (minimum of two per batch of 20 samples per matrix) should be analyzed to validate the precision of the analysis. Fortified samples (minimum of one per batch of 20 samples per

TABLE B-4. REPRODUCIBILITY OF RETENTION TIMES OF THE ORGANOCHLORINE PESTICIDES FOR 10 CONSECUTIVE INJECTIONS

Compound	Retention Time Reproducibility SD (min)[a]
alpha-BHC	0.010
beta-BHC	0.009
gamma-BHC	0.011
delta-BHC	0.011
Heptachlor	0.008
Aldrin	0.009
Heptachlor epoxide	0.009
gamma-Chlordane	0.012
Endosulfan I	0.010
4,4'-DDE	0.008
Dieldrin	0.008
Endrin	0.007
Endosulfan II	0.006
4,4'-DDD	0.008
Endrin aldehyde	0.007
Kepone	0.011
Endosulfan sulfate	0.008
4,4'-DDT	0.008
4,4'-Methoxychlor	0.007
Toxaphene	0.004-0.006[b]
PCB-1016	0.042-0.104[b]
PCB-1260	0.035-0.040[b]

SD -- Standard deviation.

[a] Number of determinations is 10 except for Kepone which is 8.

[b] Value determined for 3 major peaks for each mixture.

matrix) should be analyzed to validate the sensitivity and accuracy of the analysis. If the fortified waste samples do not indicate sufficient sensitivity to detect less than or equal to 1 µg/g of sample, then the sensitivity of the instrument should be increased or the extract should be subjected to additional cleanup. The fortified samples should be carried through all stages of the sample preparation and measurement steps. Where doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as mass spectroscopy should be used.

10.3 The analyst must, on an ongoing basis, demonstrate through the analyses of quality control check standards that the operation of the gas chromatograph is under control. The frequency of the check standard analysis should be equivalent to 10 percent of the samples analyzed. The check standard will give an indication of how good the calibration standards are. If the recovery of any compound found in the check standard is less than 80 percent, the laboratory performance is judged to be out of control, and the problem must be corrected. A new set of calibration standards should be prepared and analyzed.

10.4 The analyst must, on an ongoing basis, demonstrate through the analysis of standards that the silica gel fractionation scheme is reproducible. Batch-to-batch variation in the composition of the silica gel material and slight changes in the moisture content may cause a change in the distribution patterns of the organochlorine pesticides. Compounds found to exhibit such behavior include alpha-BHC, gamma-chlordane, 4,4'-DDT, and 4,4'-DDD.

10.5 Whenever compounds are found in more than one fraction, add up the concentrations of the various fractions if the final volumes of the fractions are identical. It is up to the analyst to decide whether the cut-off point should be 5 or 10 percent of the concentration in the fraction where the compound is expected to elute.

11. Method Performance

11.1 The MDL is defined as the minimum concentration of the test compound that can be measured and reported with 99 percent confidence that the value is above zero. The MDL concentrations listed in Table B-1 were obtained by using reagent water and sandy loam soil. Details on how to determine the MDL are given in Reference 9. The MDL actually achieved in a given analysis will vary as it is dependent on instrument sensitivity and matrix effects.

11.2 This method has been tested in a single laboratory by using clean hexane and liquid and solid waste extracts which were fortified with the test compounds at three concentrations. Single-operator precision, overall precision, and method accuracy were found to be related to the concentration of the compound and the type of matrix. For exemplification, results of the single-laboratory method evaluation are given in Tables B-5 and B-6.

TABLE B-5. ELUTION PATTERNS AND AVERAGE RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs AFTER SILICA GEL CHROMATOGRAPHY (LIQUID WASTE NO. 1 EXTRACT)

	Average ± SD (RSD)[a,b,c]				
Compound	Fraction I hexane (80 mL)	Fraction II hexane (50 mL)	Fraction III methylene chloride (15 mL)	Fraction IV ethyl acetate (50 mL)	Total recovery
alpha-BHC		57 ± 2.5 (4.4)	22 ± 9.2 (42)		79 ± 10 (13)
beta-BHC			90 ± 3.1 (3.4)		90 ± 3.1 (3.4)
gamma-BHC			90 ± 4.0 (4.4)		90 ± 4.0 (4.4)
delta-BHC			90 ± 11 (12)		90 ± 11 (12)
Heptachlor	90 ± 11 (12)				90 ± 11 (12)
Aldrin	92 ± 9.2 (10)				92 ± 9.2 (10)
Heptachlor epoxide			89 ± 4.1 (4.6)		89 ± 4.1 (4.6)
gamma-Chlordane	85 ± 7.2 (8.5)	10 ± 9.2 (92)			95 ± 8.0 (8.4)
Endosulfan I			88 ± 3.8 (4.3)		88 ± 3.8 (4.3)
4,4'-DDE	95 ± 16 (17)				95 ± 16 (17)
Dieldrin			82 ± 4.3 (5.3)		82 ± 4.3 (5.3)
Endrin			65 ± 3.1 (4.7)		65 ± 3.1 (4.7)
Endosulfan II			79 ± 7.1 (9.0)		79 ± 7.1 (9.0)
4,4'-DDD		33 ± 4.0 (15)	43 ± 16 (37)		76 ± 16 (21)
Endrin aldehyde			i		i
Kepone				113; 223[c]	113; 223[c]
Endosulfan sulfate			83 ± 4.0 (4.8)		83 ± 4.0 (4.8)
4,4'-DDT	88 ± 18 (21)				88 ± 18 (21)
4,4'-Methoxychlor			75 ± 4.6 (6.1)		75 ± 4.6 (6.1)
PCB-1016	118 ± 9.8 (8.3)				118 ± 9.8 (8.3)
PCB-1260	100 ± 18 (18)				100 ± 18 (18)

i -- Unable to determine the recovery because of interference.

[a] The values given represent the average percent recoveries from three replicate determinations ± one standard deviation; the numbers in parentheses are the relative standard deviations.

[b] The amounts spiked are 15,000, 30,000, and 150,000 ng per 2 mL extract per column for the organochlorine pesticides and PCB-1016/PCB-1260, respectively.

[c] Duplicate determinations.

TABLE B-6. ELUTION PATTERNS AND AVERAGE RECOVERIES OF THE ORGANOCHLORINE PESTICIDES AND PCBs AFTER SILICA GEL CHROMATOGRAPHY (NBS SEDIMENT EXTRACT)

Compound	Average ± SD (RSD)[a,b]				
	Fraction I hexane (80 mL)	Fraction II hexane (50 mL)	Fraction III methylene chloride (15 mL)	Fraction IV ethyl acetate (50 mL)	Total recovery
alpha-BHC		55 ± 6.1 (11)	20 ± 1.7 (8.7)		75 ± 6.0 (8.0)
beta-BHC			94 ± 3.0 (3.2)		94 ± 3.0 (3.2)
gamma-BHC			89 ± 4.1 (4.6)		89 ± 4.1 (4.6)
delta-BHC			92 ± 5.2 (5.6)		92 ± 5.2 (5.6)
Heptachlor	70 ± 7.7 (11)				70 ± 7.7 (11)
Aldrin	65 ± 4.6 (7.1)				65 ± 4.6 (7.1)
Heptachlor epoxide			91 ± 5.7 (6.3)		91 ± 5.7 (6.3)
gamma-Chlordane	71 ± 3.2 (4.5)	10 ± 2.0 (20)			81 ± 4.9 (6.1)
Endosulfan I			88 ± 5.1 (5.8)		88 ± 5.1 (5.8)
4,4'-DDE	76 ± 7.1 (9.3)				76 ± 7.1 (9.3)
Dieldrin			85 ± 9.4 (11)		85 ± 9.4 (11)
Endrin			87 ± 6.4 (7.3)		87 ± 6.4 (7.3)
Endosulfan II			81 ± 4.5 (5.5)		81 ± 4.5 (5.5)
4,4'-DDD		36 ± 2.0 (5.6)	49 ± 1.2 (2.4)		85 ± 3.1 (3.6)
Endrin aldehyde			71 ± 9.2 (13)		71 ± 9.2 (13)
Kepone				0	0
Endosulfan sulfate			86 ± 5.0 (5.8)		86 ± 5.0 (5.8)
4,4'-DDT	61 ± 7.9 (13)				61 ± 7.9 (13)
4,4'-Methoxychlor			99 ± 17 (17)		99 ± 17 (17)
PCB-1016	104 ± 2.5 (2.4)				104 ± 2.5 (2.4)
PCB-1260	95 ± 7.5 (7.9)				95 ± 7.5 (7.9)

[a] The values given represent the average percent recoveries from three replicate determinations ± one standard deviation; the numbers in parentheses are the relative standard deviations.

[b] The amounts spiked are 3,000, 6,000, and 30,000 ng per 2 mL extract per column for the organochlorine pesticides and PCB-1016/PCB-1260, respectively.

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APPENDIX C

METHOD 3510, 3520, 3540, AND 3550 PROTOCOLS (PROPOSED REVISIONS)

METHOD 3510 -- SEPARATORY FUNNEL LIQUID-LIQUID EXTRACTION

1. Scope and Application

1.1 Method 3510 is a liquid-liquid extraction procedure that uses the standard separatory funnel techniques. The sample and the extraction solvent must be immiscible to allow recovery of the target compounds. Since this method is used in conjunction with other SW-846 methods, subsequent cleanup and detection methods that will be used to analyze the extract are given elsewhere.

2. Summary of Method

A measured volume of sample is adjusted to a specific pH and is extracted with the appropriate solvent in a separatory funnel. Methylene chloride should be employed unless the method specifies a different solvent. Samples are extracted three consecutive times; the extracts are combined and dried with anhydrous sodium sulfate and are then concentrated in a Kuderna-Danish apparatus.

3. Interferences

3.1 A method blank should be performed for the compounds of interest prior to the use of this method. The level of interferences must be below the method detection limit before the actual samples are extracted.

3.2 If emulsion formation prevents acceptable extract recovery with the separatory funnel extractions, continuous liquid-liquid extraction (Method 3520) should be used.

3.3 Procedures for the removal of interfering compounds coextracted with the target compounds are described in the referring analytical methods.

4. Apparatus

4.1 Separatory funnel: 2-liter, with Teflon stopcock.

4.2 Drying column: chromatographic column approximately 400 mm long by 20 mm ID, with coarse frit. The substitution of a small pad of disposable Pyrex glass wool for the frit will help prevent cross-contamination.

4.3 Concentrator tube: Kuderna-Danish, 10 mL graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground-glass stoppers should be used to prevent evaporation of extracts.

4.4 Evaporative flask: Kuderna-Danish, 500 mL (Kontes K-5700010500 or equivalent). Attach to the concentrator tube with springs.

4.5 Snyder column: Kuderna-Danish, three-ball macro (Kontes K-503000-0121 or equivalent).

4.6 Snyder column: Kuderna-Danish, two-ball micro (Kontes K-569001-0219 or equivalent).

4.7 Vials: glass, 10 to 15 mL capacity, with Teflon-lined screw caps.

4.8 Pyrex glass wool: prerinse glass wool with appropriate solvents to ensure its cleanliness.

4 9 Silicone carbide boiling chips: approximately 10/40 mesh. Heat the chips to 400°C for 30 min or Soxhlet-extract them with methylene chloride.

4.10 Water bath: heated, with concentric ring cover, capable of temperature control (±2°C). The bath should be used in a hood.

4.11 Nitrogen evaporation device (N-Evap by Organomation Associates, Inc., South Berlin, MA, or equivalent)

4.12 pH indicator paper covering the pH range from 1 to 14.

5. Reagents

5.1 The specific reagents to be employed in this method may be listed under the referring analytical method that will be used to analyze the extract. Check the analytical method for the specific extraction solvent. If a specific extraction solvent is not listed for the compound(s) of interest, methylene chloride shall be used.

5.2 The solvent of choice should be appropriate for the method of measurement to be used, and it should give an analyte-to-solvent partition coefficient of at least 1 to 1000.

5.3 Sodium sulfate (ACS): granular anhydrous (purified by heating at 400°C for 4 hr in a shallow tray).

5.4 Sodium hydroxide (ACS): 10 N in distilled water.

5.5 Sulfuric acid (1:1): mix equal volumes of concentrated H_2SO_4 (ACS) with distilled water.

5.6 Distilled water.

5.7 Methylene chloride: pesticide quality or equivalent.

6. Sample Collection, Preservation, and Handling

6.1 Adhere to those procedures specified in the referring analytical methods for collection, preservation, and handling.

7. Sample Extraction

7.1 Use a 1-liter graduated cylinder to measure out a 1-liter portion and place it into a 2-liter separatory funnel. If less than 1 liter of sample is available, or if high concentrations are anticipated, use a smaller volume of sample.

7.2 Check the pH of the sample with wide-range pH paper and adjust it to that indicated in the referring method.

7.3 Add 60 mL of the appropriate extraction solvent to the separatory funnel and extract the sample by shaking the funnel for 2 min with periodic venting to release excess pressure.

7.4 Allow the phases to separate for a minimum of 10 min. If the emulsion interface between layers is more than one-third the size of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample but may include stirring, filtration of the emulsion through glass wool, or centrifugation.

7.5 Collect the extract into a 250-mL Erlenmeyer flask and then repeat the extraction two more times with fresh portions of solvent.

7.6 Combine the three extracts and discard the extracted waste properly if no further extractions are to be performed.

7.7 Dry the extract by passing it through a column of anhydrous sodium sulfate. Collect the dried extract in a Kuderna-Danish evaporative concentrator equipped with a 10-mL collection ampule.

7.8 Add 1 or 2 clean boiling chips to the flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL solvent to the top. Place the Kuderna-Danish apparatus on a steam or hot water bath so that the concentrator tube and the entire lower rounded surface of the flask are bathed in hot water or vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 1 mL, remove the Kuderna-Danish apparatus and allow it to drain for at least 10 min while cooling.

7.9 Remove the Snyder column and add 50 mL of the solvent specified in the referring analytical method if a solvent exchange step is required. Concentrate the extract as before. When the apparent volume of liquid reaches 1 mL, remove the Kuderna-Danish apparatus and allow it to drain and to cool for at least 10 min.

7.10 Remove the Snyder column and rinse the flask and its lower joint with 1 to 2 mL of the solvent specified in the referring analytical method into the concentrator tube.

7.11 Nitrogen blowdown technique

7.11.1 Place the concentrator tube in a warm-water bath (35°C), and evaporate the solvent to 0.5 mL by using a gentle stream of clean, dry nitrogen (filtered through a column of activated charcoal.

Caution: The extract must never be allowed to become dry.

7.11.2 Dilute the extract to 1 mL with solvent and proceed with the cleanup or analysis as described in the referring analytical method.

8. Quality Control

8.1 Comprehensive quality control procedures are specified for each target compound in the referring analytical method.

8.2 Before processing any samples, the analyst must analyze a reagent water blank to demonstrate that interferences from glassware are under control. Each time a set of samples is extracted or reagents are changed, a reagent water blank must be processed as a safeguard against laboratory contamination.

8.3 By fortifying distilled water or another liquid similar to the sample matrix, the analyst should demonstrate that the compound(s) of interest is being quantitatively recovered before applying this method to actual samples.

METHOD 3520 -- CONTINUOUS LIQUID-LIQUID EXTRACTION

1. Scope and Application

1.1 Method 3520 is a liquid-liquid extraction procedure designed to extract organic compounds from liquid samples in a continuous extraction apparatus. This method is available as an alternative to Method 3510 which is a separatory funnel extraction procedure. Compared to standard separatory funnel techniques, Method 3520 has the advantage of minimizing emulsion formation. The sample and the extraction solvent must be immiscible to allow recovery of the target compounds. Subsequent cleanup and detection methods are described in the referring analytical methods.

1.2 Method 3520 is designed for extraction solvents with greater density than the sample. Continuous extraction devices are available for extraction solvents that are less dense than the sample. The analyst must demonstrate the effectiveness of any such automatic extraction device before employing it in sample extraction.

2. Summary of Method

A measured volume of sample is adjusted to a specific pH and is placed into a continuous liquid-liquid extractor. The extraction pH and solvent are those specified in the referring analytical method. Samples are extracted for 16 hours; the extract is collected, dried with anhydrous sodium sulfate, and concentrated in a Kuderna-Danish apparatus. In some cases, the sample pH is adjusted after the first extraction, and continuous extraction is carried out for another 16 hours to recover an additional class of compounds.

3. Interferences

3.1 A method blank should be performed for the compounds of interest prior to the use of this method. The level of interferences must be below the method detection limit before the actual samples are extracted.

3.2 Procedures for the removal of interfering compounds coextracted with the target compounds are described in the referring analytical methods.

4. Apparatus

4.1 A continuous liquid-liquid extractor equipped with Teflon or glass connecting joints and stopcocks requiring no lubrication (Hershberg-Wolfe type, Ace Glass Company, Vineland, NJ, P/N 6841-10, equivalent).

4.2 Drying column: chromatographic column approximately 400 mm long by 20 mm ID, with coarse frit. Substitution of a small pad of disposable Pyrex glass wool for the frit will help prevent cross-contamination.

4.3 Concentrator tube: Kuderna-Danish, 10 mL graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground-glass stoppers should be used to prevent evaporation of extracts.

4.4 Evaporative flask: Kuderna-Danish, 500 mL (Kontes K-5700010500 or equivalent). Attach to the concentrator tube with springs.

4.5 Snyder column: Kuderna-Danish, three-ball macro (Kontes K-503000-0121 or equivalent).

4.6 Snyder column: Kuderna-Danish, two-ball micro (Kontes K-569001-0219 or equivalent).

4.7 Vials: glass, 10 to 15 mL capacity, with Teflon-lined screw caps.

4.8 Pyrex glass wool: prerinse glass wool with appropriate solvents to ensure its cleanliness.

4 9 Silicone carbide boiling chips: approximately 10/40 mesh. Heat them to 400°C for 30 min or Soxhlet-extract them with methylene chloride.

4.10 Water bath: heated, with concentric ring cover, capable of temperature control (±2°C). The bath should be used in a hood.

4.11 Nitrogen evaporation device (N-Evap by Organomation Associates, Inc., South Berlin, MA, or equivalent)

4.12 pH indicator paper covering the pH range from 1 to 14.

5. Reagents

5.1 The specific reagents to be employed in this method may be listed under the referring analytical method that will be used to analyze the extract. Check the analytical method for the specific extraction solvent. If a specific extraction solvent is not listed for the compound(s) of interest, methylene chloride shall be used.

5.2 The solvent of choice should be appropriate for the method of measurement to be used, and it should give an analyte-to-solvent partition coefficient of at least 1 to 1000.

5.3 Sodium sulfate (ACS): granular anhydrous (purified by heating at 400°C for 4 hours in a shallow tray).

5.4 Sodium hydroxide (ACS): 10 N in distilled water.

5.5 Sulfuric acid (1:1): mix equal volumes of concentrated H_2SO_4 (ACS) with distilled water.

5.6 Distilled water.

5.7 Methylene chloride: pesticide quality or equivalent.

6. Sample Collection, Preservation, and Handling

6.1 Adhere to those procedures specified in the referring analytical methods for collection, preservation, and handling.

7. Sample Extraction

7.1 Use a 1-liter graduated cylinder to measure out a 1-liter portion and place it into a continuous extractor. If less than 1 liter of sample is available, or if high concentrations are anticipated, use smaller volumes of sample and add organics-free water to bring the sample volume to 1 liter.

7.2 Refer to Figure C-1 to aid in understanding the procedures described in Sections 7.3 through 7.7 for setting up the continuous extractor. The analyst is reminded that the following steps apply to sample/solvent systems where the solvent is more dense than the sample.

7.3 Place 150 mL of the extraction solvent in the extractor and 350 mL of the extracting solvent in the 500-mL distilling flask. Add several boiling chips to the distilling flask.

7.4 Add the sample to the continuous extractor. More organics-free water may be needed to ensure proper operation.

7.5 Turn on the cooling water and the heating mantle and extract the sample for 16 hours.

7.6 Let the system cool and remove the extract contained in the 500-mL distilling flask.

7.7 If an additional extraction is to be performed, adjust the sample pH accordingly. Attach a 500-mL distilling flask containing 350 mL of extraction solvent, add several boiling chips, and proceed from step 7.5.

7.8 Dry the extract by passing it through a column of anhydrous sodium sulfate. Collect the dried extract in a Kuderna-Danish evaporative concentrator equipped with a 10-mL collection ampule.

7.9 Add 1 or 2 clean boiling chips to the flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL solvent to the top. Place the Kuderna-Danish apparatus on a steam or hot-water bath so that the concentrator tube and the entire lower rounded surface of the flask are bathed in hot water or vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 1 mL, remove the Kuderna-Danish apparatus and allow it to drain for at least 10 min while cooling.

7.10 Remove the Snyder column and add 50 mL of the solvent specified in the analytical method if a solvent exchange step is required. Concentrate

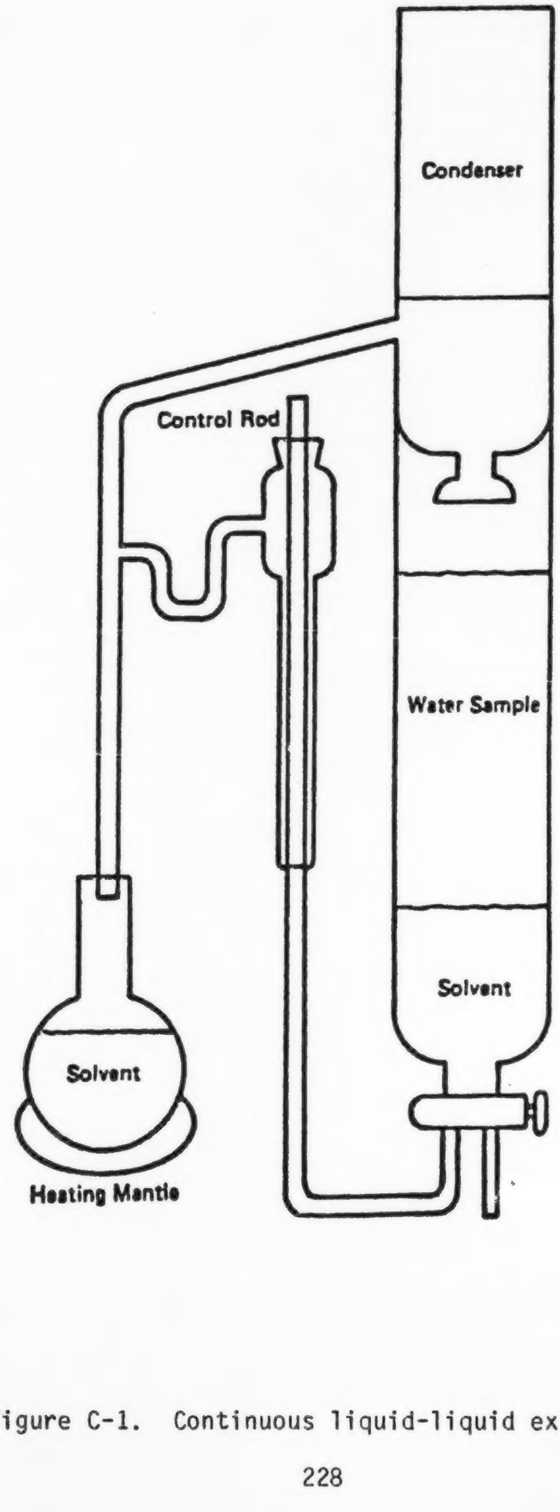
Condenser
Control Rod
Water Sample
Solvent
Solvent
Heating Mantle

Figure C-1. Continuous liquid-liquid extractor.

the extract as before. When the apparent volume of liquid reaches 1 mL, remove the Kuderna-Danish apparatus and allow it to drain and to cool for at least 10 min.

7.11 Remove the Snyder column and rinse the flask and its lower joint with 1 to 2 mL of the solvent specified in the referring analytical method into the concentrator tube.

7.12 Nitrogen blowdown technique

7.12.1 Place the concentrator tube in a warm-water bath (35°C), and evaporate the solvent to 0.5 mL by using a gentle stream of clean, dry nitrogen (filtered through a column of activated charcoal).

Caution: The extract must never be allowed to become dry.

7.12.2 Dilute the extract to 1 mL with solvent and proceed with the cleanup or analysis as described in the referring analytical methods.

8. Quality Control

8.1 Comprehensive quality control procedures are specified for each target compound in the referring analytical method.

8.2 Before processing any samples, the analyst must analyze a reagent water blank to demonstrate that interferences from glassware are under control. Each time a set of samples is extracted or reagents are changed, a reagent water blank must be processed as a safeguard against laboratory contamination.

8.3 By fortifying distilled water or another liquid similar to the sample matrix, the analyst should demonstrate that the compound(s) of interest is being quantitatively recovered before applying this method to actual samples.

METHOD 3540 -- SOXHLET EXTRACTION

1. Scope and Application

Method 3540 is a procedure for extracting organic compounds from solids such as soils and sludges. The Soxhlet extraction procedure ensures intimate contact of the sample matrix with the extraction solvent. Since this method is used in conjunction with other SW-846 methods, subsequent cleanup and detection methods that will be used to analyze the extract are given elsewhere.

2. Summary of Method

A known amount of the solid sample is mixed with anhydrous sodium sulfate, is placed in an extraction thimble or between two plugs of glass wool, and is extracted with an appropriate solvent in a Soxhlet extractor. The extract is then dried and concentrated and is either cleaned-up further or is analyzed directly by use of the referring analytical method.

3. Interferences

3.1 A method blank should be performed for the compounds of interest prior to the use of this method. The level of interferences must be below the method detection limit before the actual samples are extracted.

3.2 Procedures for the removal of interfering compounds coextracted with the target compounds are described in the referring analytical method that will be used to analyze the extract.

4. Apparatus

4.1 Soxhlet extractor: 40 mm ID, with 500-mL round bottom flask.

4.2 Rheostat-controlled heating mantle.

4.3 Drying column: chromatographic column approximately 400 mm long x 20 mm ID, with coarse frit. Substitution of a small pad of disposable Pyrex glass wool for the frit will prevent cross-contamination.

4.4 Concentrator tube: Kuderna-Danish, 10 mL graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground-glass stoppers should be used to prevent evaporation of extracts.

4.5 Evaporative flask: Kuderna-Danish, 500 mL (Kontes K-5700010500 or equivalent). Attach to the concentrator tube with springs.

4.6 Snyder column: Kuderna-Danish, three-ball macro (Kontes K-503000-0121 or equivalent).

4.7 Snyder column: Kuderna-Danish, two-ball micro (Kontes K-569001-0219 or equivalent).

4.8 Vials: glass, 10 to 15 mL capacity, with Teflon-lined screw caps.

4.9 Pyrex glass wool: prerinse glass wool with appropriate solvents to ensure its cleanliness.

4.10 Silicone carbide boiling chips: approximately 10/40 mesh. Heat them to 400°C for 30 min or Soxhlet-extract them with methylene chloride.

4.11 Water bath: heated, with concentric ring cover, capable of temperature control (±2°C). The bath should be used in a hood.

4.12 Nitrogen evaporation device (N-Evap by Organomation Associates, Inc., South Berlin, MA, or equivalent).

4.13 Apparatus for determining percent moisture.

4.13.1 Drying oven

4.13.2 Desiccator.

4.13.3 Porcelain crucibles.

4.14 Vacuum filtration apparatus.

4.14.1 Buchner funnel.

4.14.2 Filter paper, Whatman No. 41, or equivalent.

4.15 Amber bottles, 250 mL.

5. Reagents

5.1 The specific reagents to be employed in this method may be listed under the referring analytical methods that will be used to analyze the extract. Check the analytical method for the specific extraction solvent.

5.2 Sodium sulfate (ACS): granular anhydrous (purified by heating at 400°C for 4 hours in a shallow tray).

5.3 Solvent(s): soil samples shall be extracted by using either of the following solvent systems.

5.3.1 Acetone/hexane, 1:1 v/v, ACS reagent grade only.

5.3.2 Toluene/methanol, 10:1 v/v, ACS reagent grade only.

6. Sample Collection, Preservation, and Handling

6.1 Adhere to those procedures specified in the referring analytical methods for collection, preservation, and handling.

7. Procedure

7.1 Blend 10 g of the solid sample with an equal weight of anhydrous sodium sulfate and place the mixture in either a glass or paper extraction thimble. The extraction thimble must drain freely for the duration of the extraction period. The use of a glass-wool plug above and below the sample is also acceptable.

7.2 Place 300 mL of the extraction solvent into a 500-mL round-bottom flask containing a few boiling chips. Attach the flask to the extractor, and extract the solids for 16 hours.

7.3 Allow the extract to cool after the extraction is complete. Rinse the condensor with the extraction solvent and drain the Soxhlet apparatus into the collecting round-bottom flask. Filter the extract and dry it by passing it through a 4-in column of sodium sulfate which has been washed with the extraction solvent. Collect the dried extract in a 500-mL Kuderna-Danish (K-D) flask fitted with a 10-mL graduated concentrator tube. Wash the extractor flask and sodium sulfate column with 100 to 125 mL of the extraction solvent.

7.4 Add 1 or 2 clean boiling chips to the flask, and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL solvent to the top. Place the Kuderna-Danish apparatus on a steam- or hot-water bath so that the concentrator tube and the entire lower rounded surface of the flask are bathed in hot water or vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 1 mL, remove the Kuderna-Danish apparatus and allow it to drain for at least 10 min while cooling.

7.5 Remove the Snyder column and add 50 mL of the solvent specified in the analytical method if a solvent exchange step is required. Concentrate the extract as before. When the apparent volume of liquid reaches 1 mL, remove the Kuderna-Danish apparatus and allow it to drain and to cool for at least 10 min.

7.6 Remove the Snyder column and rinse the flask and its lower joint with 1 to 2 mL of the solvent specified in the referring analytical method into the concentrator tube.

7.7 If GPC cleanup is not required, proceed to Paragraph 7.8.; if GPC is required, refer to Method 8080 for instructions on how to perform it.

7.8 Nitrogen blowdown technique

7.8.1 Place the concentrator tube in a warm-water bath (35°C), and evaporate the solvent to 0.5 mL by using a gentle stream of clean, dry nitrogen (filtered through a column of activated charcoal).

Caution: The extract must never be allowed to become dry.

7.8.2 Dilute the extract to 1 mL with solvent and proceed with the cleanup or analysis as described in the referring analytical method.

7.9 Moisture determination: Immediately after weighing the sample for extraction, weigh 5 to 10 g of the sample into a tared crucible. Determine the percent moisture by drying overnight at 105°C. Allow to cool in a desiccator before weighing. Concentrations of individual analytes will be reported relative to the dry weight of soil or sediment.

$$\frac{\text{weight of sample} - \text{weight of dry sample}}{\text{weight of sample}} \times 100 = \text{percent moisture}$$

8. Quality Control

8.1 Comprehensive quality control procedures are specified for each target compound in the referring analytical method.

8.2 Before processing any samples, the analyst must perform a method blank to demonstrate that interferences from glassware are under control. Each time a set of samples is extracted or reagents are changed, a method blank must be performed as a safeguard against laboratory contamination.

8.3 By fortifying clean soil or another solid similar to the sample matrix, the analyst should demonstrate that the compound(s) of interest is being quantitatively recovered before applying this method to actual samples.

METHOD 3550 -- SONICATION EXTRACTION

1. Scope and Application

1.1 Method 3550 is a procedure for extracting organic compounds from solids such as soils and sludges. The sonication process ensures intimate contact of the sample matrix with the extraction solvent. Since this method is used in conjunction with other SW-846 methods, subsequent cleanup and detection methods that will be used to analyze the extract are given elsewhere.

2. Summary of Method

A known amount of sample of the solid waste is ground, is mixed with the extraction medium, and then is dispersed into the solvent by using sonication. The extract is dried with anhydrous sodium sulfate and is concentrated in a Kuderna-Danish apparatus. The resulting solution may be cleaned-up further or may be analyzed directly by using the appropriate technique.

3. Interferences

3.1 A method blank should be performed for the compounds of interest prior to the use of this method. The level of interferences must be below the method detection limit before the actual samples are extracted.

3.2 Procedures for the removal of interfering compounds coextracted with the target compounds are described in the referring analytical method that will be used to analyze the extract.

4. Apparatus and Materials

4.1 Apparatus for grinding: if the sample will not pass through a 1-mm standard sieve or cannot be extruded through a 1-mm opening, it should be processed into a homogeneous sample that meets these requirements. Fisher Mortar Model 155 Grinder, Fisher Scientific Co., Catalogue Number 8-323, or an equivalent brand and model, is recommended for sample processing. This grinder should handle most solid samples except gummy, fibrous, or oily materials.

4.2 Sonication: a horn-type sonicator equipped with a titanium tip should be used. The following sonicators, or equivalent brands and models, are recommended: Sonifer/Cell Disruptor, Model W-350, Ultrasonics Inc., or Sonic Dismembrator, Model 300, Fisher Scientific Co., Catalog Number 15-338-40.

4.3 Drying column: chromatographic column approximately 400 mm long by 20 mm ID, with coarse frit. Substitution of a small pad of disposable Pyrex glass wool for the frit will help prevent cross-contamination.

4.4 Concentrator tube: Kuderna-Danish, 10 mL graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground-glass stoppers should be used to prevent evaporation of extracts.

4.5 Evaporative flask: Kuderna-Danish, 500 mL (Kontes K-5700010500 or equivalent). Attach to the concentrator tube with springs.

4.6 Snyder column: Kuderna-Danish, three-ball macro (Kontes K-503000-0121 or equivalent).

4.7 Snyder column: Kuderna-Danish, two-ball micro (Kontes K-569001-0219 or equivalent).

4.8 Vials: glass, 10 to 15 mL capacity, with Teflon-lined screw caps.

4.9 Pyrex glass: prerinse glass wool with appropriate solvents to ensure its cleanliness.

4.10 Silicone carbide boiling chips: approximately 10/40 mesh. Heat them to 400°C for 30 min or Soxhlet-extract them with methylene chloride.

4.11 Water bath: heated, with concentric ring cover capable of temperature control (±2°C). The bath should be used in a hood.

4.12 Nitrogen evaporation device (N-Evap by Organomation Associates, Inc., South Berlin, MA, or equivalent)

4.13 Apparatus for determining percent moisture.

4.13.1 Drying oven.

4.13.2 Desiccator.

4.13.3 Porcelain crucibles.

4.14 Vacuum filtration apparatus.

4.14.1 Buchner funnel.

4.14.2 Filter paper, Whatman No. 41, or equivalent.

4.15 Amber bottles, 250 mL.

5. Reagents

5.1 The specific reagents to be employed in this method may be listed under the referring analytical method that will be used to analyze this extract. Check the analytical method for the specific extraction solvent. If a specific extraction solvent is not listed for the compound(s) of interest, methylene chloride shall be used.

5.2 Sodium sulfate (ACS): granular anhydrous (purified by heating at 400°C for 4 hours in a shallow tray).

5.3 Sodium hydroxide (ACS): 10 N in distilled water.

6. Sample Collection, Preservation, and Handling

6.1 Adhere to those procedures specified in the referring analytical methods for collection, preservation, and handling.

7. Sample Extraction

7.1 Grind or subdivide the solid sample so that it either passes through a 1-mm sieve or can be extruded through a 1-mm hole. Introduce sufficient sample into the grinding apparatus to yield at least 10 g after grinding.

7.2 Transfer 50 g of the soil/sediment to a 100-mL beaker. Add 50 mL water and stir for 1 hr. Determine the pH of the sample with a glass electrode and a pH meter while stirring.

7.3 Weigh 10.0 g of suitably dispersed material into a 75-mL glass flask, adjust the pH as required, and add 30 mL of an appropriate solvent. Sonicate with agitation for approximately 3 min. Filter the resulting suspension by using vacuum filtration, or centrifuge and decant the extract. Reextract the solid residue with an additional 30-mL portion of solvent. Repeat the extraction a third time and use fresh solvent each time.

7.4 After the extraction is complete, filter the extract and dry it by passing it through a 4-in column of sodium sulfate which has been washed with the extraction solvent. Collect the dried extract in a 500-mL Kuderna-Danish flask fitted with a 10-mL graduated concentrator tube and a three-ball Snyder column. Wash the extractor flask and sodium sulfate column with 100 to 125 mL of the extraction solvent.

7.5 Add 1 or 2 clean boiling chips to the flask, and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL solvent to the top. Place the Kuderna-Danish apparatus on a steam or hot water bath so that the concentrator tube and the entire lower rounded surface of the flask are bathed in hot water or vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 1 mL, remove the Kuderna-Danish apparatus and allow it to drain for at least 10 min while cooling.

7.6 Remove the Snyder column and add 50 mL of the solvent specified in the analytical method if a solvent exchange step is required. Concentrate the extract as before. When the apparent volume of liquid reaches 1 mL, remove the Kuderna-Danish apparatus and allow it to drain and to cool for at least 10 min.

7.7 Remove the Snyder column, rinse the flask and its lower joint with 1 to 2 mL of the solvent specified in the referring analytical method into the concentrator tube.

7.8 If GPC cleanup is not required, proceed to paragraph 7.9; if GPC cleanup is required, refer to Method 8080 for instructions on how to perform it.

7.9 Nitrogen blowdown technique.

7.9.1 Place the concentrator tube in a warm-water bath (35°C), and evaporate the solvent to 0.5 mL by using a gentle stream of clean, dry nitrogen (filtered through a column of activated charcoal.

Caution: The extract must never be allowed to become dry.

7.9.2 Dilute the extract to 1 mL with solvent and proceed with the cleanup or analysis as described in the referring analytical method.

7.10 Moisture determination: Immediately after weighing the sample for extraction, weigh 5 to 10 g of the sample into a tared crucible. Determine the percent moisture by drying overnight at 105°C. Allow to cool in a desiccator before weighing. Concentrations of individual analytes will be reported relative to the dry weight of soil or sediment.

$$\frac{\text{weight of sample - weight of dry sample}}{\text{weight of sample}} \times 100 = \text{percent moisture}$$

8. Quality Control

8.1 Comprehensive quality control procedures are specified for each target compound in the referring analytical method.

8.2 Before processing any samples, the analyst must analyze a solvent blank to demonstrate that interferences from glassware are under control. Each time a set of samples is extracted or reagents are changed, a solvent blank must be processed as a safeguard against laboratory contamination.

8.3 By fortifying clean soil or another solid similar to the sample matrix, the analyst should demonstrate that the compound(s) of interest is being quantitatively recovered before applying this method to actual samples.